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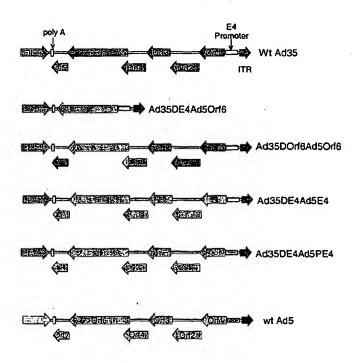
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[Continued on next page]

(54) Title: METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY



(57) Abstract: Various methods propagating and rescuing multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line are Typically, replication-defective disclosed. adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The disclosed methods offer the ability to propagate vectors derived from multiple adenoviral serotypes in a single production cell line which expresses E1 proteins from a single serotype. Propagation in this manner is accomplished by providing all or a portion of an E4 region in cis within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the El gene product(s) of the complementing cell line. Interaction between the expressed E1 of the cell line and the heterologous E4 of the replication-defective adenoviral vectors enables their propagation and rescue. The invention bypasses a need in the art to customize specific cell lines to the serotype or subgroup of the adenoviral vector being propagated and enables one to easily and rapidly develop alternative adenoviral serotypes as gene delivery vectors for use as vaccines or as a critical component in gene therapy.

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TITLE OF THE INVENTION

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METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of application serial nos. 60/458,825, filed March 28, 2003; 60/455,312, filed March 17, 2003; 60/455,234, filed March 17, 2003; and 60/405,182, filed August 22, 2002.

FIELD OF THE INVENTION

The present invention concerns various methods to propagate and rescue multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line. Typically, replication-defective adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The methods disclosed herein offer the ability to propagate vectors derived from multiple serotypes in a single cell line expressing E1 proteins from a single serotype. Such propagation of a wide range of vectors in one cell line is accomplished by providing all or a portion of an E4 region in cis within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the E1 gene product(s) of the complementing cell line. Interaction between the E1 gene products of the cell line and the heterologous E4 gene products of the replication-defective adenoviral vector enables the propagation and rescue of the recombinant replication-defective adenovirus vectors. The invention, therefore, bypasses an existing need in the art to customize complementing cell lines to the specific serotype or subgroup of the adenoviral vector being propagated or, alternatively, to have to transfect a cell line with an E4 region and then regulate the expression in trans of the E4 region within the E1 complementing cell line.

BACKGROUND OF THE INVENTION

Beginning with the first human adenoviruses (Ads) isolated over four decades ago (Rowe et al., Proc. Soc. Exp. Biol. Med., 84:570-579, 1953), over 100 distinct serotypes of adenovirus have been isolated which infect various mammalian species, 51 of which are of human origin (Straus, Adenovirus infections in humans. In The Adenoviruses. 451-498, 1984; Hierholzer et al., J. Infect. Dis., 158: 804-813, 1988; Schnurr and Dondero, Intervirology., 36: 79-83, 1993; Jong et al., J Clin Microbiol., 37:3940-3945:1999). The human serotypes have been categorised into six subgenera (A-F) based on a number of biological, chemical, immunological and structural criteria; criteria which include hemagglutination properties of rat

and rhesus monkey erythrocytes, DNA homology, restriction enzyme cleavage patterns, percentage of G+C content and oncogenicity (Straus, Adenovirus infections in humans. In *The Adenoviruses*. 451-498, 1984; Horwitz, Adenoviridae and their replication, *In Virology*: 1679-172, 1990).

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Deletion of an essential E1 region common to the various adenovirus serotypes has enabled the use of adenovirus vectors as gene transfer vectors for vaccine and gene therapy purposes. Resultant replication-defective vectors are propagated in cell lines that provide the deleted E1 gene products in trans. Supplementation of the essential E1 gene products in trans in this manner works well when the E1 gene products are from the same or a highly similar serotype. As such, E1-deleted group C serotypes (Ad1, Ad2, Ad5 and Ad6) grow well in 293 or PER.C6 cells which contain and express the Ad5 E1 region. In contrast, E1-deleted serotypes other than group C, for example those from subgroups A, B, D, E, and F (e.g., Ad3, Ad4, and Ad7 to Ad51), do not replicate efficiently in 293 or PER.C6 cells. The Ad5 E1 sequences in 293 and PER.C6 cells do not fully complement the replication of these alternative serotypes. This presents a challenge due to the fact that the most characterized and studied complementing cell lines available for growth and propagation of adenovirus are based on E1 sequence from adenovirus serotype 5.

This inability to fully complement the replication of serotypes other than group C adenovirus in Ad5 E1 complementing cell lines has been attributed to the inability of Ad5 (group C) E1b 55K gene product to functionally interact with the E4 gene products of non-group C serotypes. While the interaction is conserved within members of the same subgroup, it is not well conserved between subgroups.

Hence, cell lines expressing both Ad5 E1 and ORF6 were generated and proved useful in complementing alternative adenovirus serotypes; see, e.g., Abrahamsen et al., 1997 J. Virol. 8946-8951. Such incorporation of E4 (or ORF6) into Ad 5 complementing cell lines as was done in Abrahamsen et al., supra, is known.

U.S. Patent No. 5,849,561 discloses complementation of an E1-deleted non-group C adenovirus vector in an Ad5-E1 complementing cell line which also expresses portions of the Ad5-E4 gene.

U.S. Patent No. 6,127,175, issued to Vigne, *et al.*, discloses a stably transfected mammalian cell line which expresses a portion of the E4 region of adenovirus, preferably ORF6 or ORF6/7. Such a cell line is useful for complementation of recombinant Ad genomes deficient in the E4 region.

European Application EP 1 054 064 A1 discloses recombinant, replication deficient adenovirus 35 (Ad35) vectors and cell lines which complement in trans the growth of

these vectors. A cell line which expresses Ad5E1A and E2A genes (PER.C6) was shown to complement an Ad35-E1 deleted vector upon co-expression of Ad35-E1B proteins.

U.S. Patent No. 6,270,996, issued to Wilson, et al., discloses E1/E4 deleted adenovirus vectors and E1/E4(ORF6) cell lines which complement in trans virus growth without resulting in cell toxicity.

U.S. Patent No. 6,202,060, issued to Mehtali, et al., discloses adenoviral vectors wherein portions of the early genes are under control of an inducible promoter. The '060 patent also discloses complementing cell lines which may be used in tandem with these Ad vectors.

The generation of serotype-specific cell lines providing a complementing serotype-specific E1 gene product(s) in trans is known as well.

Although Ad5-based vectors have been used extensively in a number of gene therapy trials, there may be limitations on the use of Ad5 and other group C adenoviral vectors due to preexisting immunity in the general population due to natural infection. Ad5 and other group C members tend to be among the most seroprevalent serotypes. Immunity to existing vectors may develop as a result of exposure to the vector during treatment. These types of preexisting or developed immunity to seroprevalent gene delivery vectors may limit the effectiveness of gene therapy or vaccination efforts. Alternative adenovirus serotypes, thus, constitute very important targets in the pursuit of gene delivery systems capable of evading the host immune response.

There remains both a practical and commercial need for an adenovirus-based vaccine and/or gene therapy delivery system which allows for the production of multiple serotype recombinant adenovirus vectors in a single source complementing mammalian cell line. The present invention addresses and overcomes this deficiency in the art by disclosing novel methods for propagating multiple serotype recombinant Ad vectors in a single complementing cell line where the required serotype-specific sequences are provided *in cis*.

SUMMARY OF THE INVENTION

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The present invention relates to an enhanced means for propagating replication-defective adenovirus in an E1-complementing cell line(s) where the E1 gene product(s) being expressed is not native to the adenovirus being propagated. The method is based on Applicants' finding that supply, in cis, of a nucleic acid sequence encoding all or a portion of a heterologous adenoviral E4 region which is native to a virus of the same or highly similar serotype as the E1 gene product(s) of the complementing cell line enables the growth of adenoviral vectors of varying serotype in any single complementing cell line, despite the fact the cell line is not customized for the particular serotype of vector being propagated. This is of particular

importance given that existing and settled adenoviral E1-complementing cell lines (such as PER.C6™ and 293) are based on one of the most prominent adenovirus serotypes (Ad5) and are not suited for the large-scale propagation and rescue of alternative serotypes.

The basic steps involved in the propagation of adenoviral vectors in accordance with the methods of the instant invention are as follows: First, all or a portion of a heterologous adenoviral E4 region comprising nucleic acid sequence encoding at least open reading frame 6 (ORF6) is inserted into a replication-defective adenoviral vector. By "heterologous", Applicants mean that the nucleic acid sequence is not native to the viral vector being propagated, i.e., not normally present within a virus of the same or highly similar serotype. As will be described, the adenoviral E4 region or portion thereof can be either a nucleic acid sequence encoding ORF 6 or any larger portion of the E4 region, and includes nucleic acid comprising the complete E4 region with E4 promoter. The region into which the nucleic acid is incorporated is not limited, i.e., the insertion can be made into the complete E4 region with E4 promoter or into a smaller portion narrowing into the ORF6 region. Alternatively, the heterologous E4 region or portion thereof can be inserted into different areas of the genome such as the E1 or E3 regions. Further, the native E4 region or portion thereof can be deleted and replaced, or left intact. This is not deemed a critical element of the instant invention. What is a critical element is that the heterologous E4 region or portion thereof being inserted is native to a virus of the same or highly similar serotype as the E1 gene product(s) expressed by the complementing cell line.

Following the modification of the adenoviral vector of interest, the recombinant adenovirus is then introduced into an adenoviral E1-complementing cell line and allowed to propagate. The adenovirus is subsequently harvested and rescued from the complementing cell line.

The resultant virus can be studied and used in various gene therapy and vaccine efforts. The virus, therefore, forms an important aspect of the instant invention.

BRIEF DESCRIPTION OF THE DRAWINGS

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FIGURE 1 illustrates a transcription map for adenovirus serotype 5. The linear genome is divided into 100 map units as well as into r- and l- strands which designate the direction of transcription. Early transcription units are designated with an E and are active prior to viral DNA replication. Late transcription units are designated with and L and are active primarily after DNA replication. Promoters are represented as brackets and polyadenylation sites as arrowheads. The tripartite leader is designated 1, 2, and 3.

FIGURES 2A-1 through 2A-10 illustrate the nucleic acid sequence for the wild-type adenovirus 35 (SEQ ID NO: 1) utilized in the Examples.

FIGURE 3 illustrates the homologous recombination scheme utilized to recover pAd35 Δ E1.

FIGURE 4 illustrates the various configurations of the E4 regions (or portions) within the alternative serotype recombinants.

FIGURE 5 illustrates the homologous recombination scheme utilized to recover pAd35 Δ E1 Δ E4Ad5Orf6.

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FIGURE 6 illustrates the nucleic acid sequence encoding the gag expression cassette (SEQ ID NO: 2). The various regions of the figure are as follows: (1) a first underlined segment of nucleic acid sequence encoding the immediate early gene promoter region from human cytomegalovirus; (2) a first segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; (3) a region in caps which contains the coding sequence of HTV-1 gag; (4) a second segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; and (5) a second underlined segment, this segment containing nucleic acid sequence encoding a bovine growth hormone polyadenylation signal sequence.

FIGURE 7 illustrates the nucleic acid sequence encoding the SEAP expression cassette (SEQ ID NO: 3). The various regions of the figure are as follows: (1) a first underlined segment of nucleic acid sequence encoding the immediate early gene promoter region from human cytomegalovirus; (2) a first segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; (3) a region in caps which contains the coding sequence of the human placental SEAP gene; (4) a second segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; and (5) a second underlined segment, this segment containing nucleic acid sequence encoding a bovine growth hormone polyadenylation signal sequence.

FIGURE 8 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10¹⁰ vp doses of Ad35 vectors. This experiment was designed to address any effects of E3 deletion. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Shown are geometric means for each cohort of 5 mice.

vp doses of Ad35 vectors. This experiment was designed to address any effects of Ad5 sequence insertion into the Ad35 genome. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Two extra cohorts received 10^10 vp and 10^9 vp of Ad5 vector. Shown are geometric means for each cohort of 5 mice.

FIGURES 10A-B illustrate in vivo SEAP expression using MRKAd5-based (A) and Ad35ΔE1ΔE4Ad5Orf6-based (B) vector in rhesus macaques. Shown are the serum antigen

levels for individual monkeys following a single intramuscular (i.m.) injection of 10¹¹ vp MRKAd5SEAP (filled circles), 10⁹ vp MRKAd5SEAP (open boxes) or 10¹¹ vp Ad35ΔE1SEAPΔE4Ad5Orf6.

FIGURE 11 illustrates in vivo SEAP expression in African green monkeys using Ad5- and Ad35-based vectors. Shown are the antigen levels for each animal in serum samples collected two days after the treatment.

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FIGURE 12 illustrates the homologous recombination scheme utilized to recover $pAd24\Delta E1$.

FIGURE 13 illustrates the homologous recombination scheme utilized to recover pAd24ΔE1Ad5Orf6.

FIGURE 14 illustrates the configuration of E4 regions in the Ad24 recombinants generated.

FIGURE 15 illustrates the growth kinetics of the Ad24-based vectors in PER.C6 cells.

FIGURES 16A-1 through 16A-10 illustrate the nucleic acid sequence for wild-type adenovirus serotype 24 (SEQ ID NO: 5). The ATCC product number for Ad24 is VR-259.

FIGURE 17 illustrates, in tabular format, gag-specific T cell responses in monkeys immunized with MRKAd5-HIVgag and Ad24 HIV vectors. Shown are the numbers of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag peptide pool. The pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

FIGURE 18 illustrates, in tabular format, the characterization of the gag-specific T cells in monkeys immunized with 10^11 vp of MRKAd5-HIV1gag and Ad24ΔE1gagΔOrf6Ad5Orf6. Shown are the percentages of CD3+ T cells that are either gag-specific CD4+ or gag-specific CD8+ cells. These values were corrected for mock values (<0.03%).

FIGURE 19 illustrates individual anti-p24 titers (in mMU/mL) in macaques immunized with gag-expressing adenovirus vectors.

FIGURE 20 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^10 vp doses of Ad24 vectors. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Two extra cohorts received 10^10 vp and 10^9 vp of Ad5 vector. Shown are geometric means for each cohort of 5 mice.

FIGURE 21 illustrates in vivo SEAP expression using MRKAd5 and Ad24 vectors in rhesus macaques. Shown are the geometric means of the SEAP levels for cohorts of 3 monkeys. In bars are the standard errors of the geometric means.

FIGURE 22 illustrates a homologous recombination scheme to be utilized to recover pAd24ΔE1ΔE4Ad5Orf6.

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FIGURE 23 illustrates gag-specific T cell responses in rhesus macaques immunized following a heterologous Ad5/Ad6 prime-Ad24 boost regimen. a: Mock, no peptide: gag, 20-mer peptide pool encompassing entire gag sequence; b: Peak response after 2 or 3 doses of the priming vaccine; c: 3 wks prior to boost; d: 4 wks after boost; e: ND, not determined.

FIGURE 24 illustrates, in tabular format, the percentages of CD3⁺ T lymphocytes that are gag-specific CD8⁺ cells or gag-specific CD4⁺ cells determined after the Ad24 Boost Immunization (wk 60). Numbers reflect the percentages of circulating CD3+ lymphocytes that are either gag-specific CD4+ or gag-specific CD8+ cells. Mock values (equal to or less than 0.01%) have been subtracted.

FIGURE 25 illustrates gag-specific T cell responses in rhesus macaques immunized following a heterologous Ad 24 prime-Ad5 boost regimen. a: Mock, no peptide: gag, 20-mer peptide pool encompassing entire gag sequence; b: Peak response after 2 doses of the priming vaccine; c: Wk 24; d: 4 wks after boost; e: ND, not determined.

FIGURE 26 illustrates the homologous recombination scheme utilized to recover pAd34 Δ E1 Δ E4Ad5Orf6.

FIGURE 27 illustrates the homologous recombination scheme utilized to recover pMRKAd34ΔE1ΔE4Ad5Orf6.

FIGURES 28A-1 to 28A-9 illustrate a nucleic acid sequence for wild-type adenovirus serotype 34 (SEQ ID NO: 12). The ATCC product number for Ad34 is VR-716.

FIGURE 29 illustrates the time course of SEAP expression using MRKAd5 and Ad34 vectors in rhesus macaques. Data represent cohort geometric means.

FIGURE 30 illustrates, in tabular format, T cell responses induced using MRKAd5 and Ad34 vectors expressing HIV-1 gag. Data are expressed in numbers of spot-forming cells per million PBMC (SFC/10^6 PBMC). "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 31 illustrates, in tabular format, the levels of CD4+ and CD8+ Gagspecific T cells in Ad34-immunized macaques at week 12. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 32 illustrates, in tabular format, T cell responses induced using a heterologous Ad34 prime/Ad35 boost regimen in macaques. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 33 illustrates, in tabular format, the levels of CD4+ and CD8+ Gag-specific T cells in Ad34 primed/Ad35 boosted macaques at week 28. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

DETAILED DESCRIPTION OF THE INVENTION

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The present invention details an efficient strategy for the propagation and rescue of alternative adenoviral serotypes utilizing available adenovirus production cell lines, nullifying the need to customize available cell lines for a specific serotype of interest. This is enabled by the incorporation of a critical E4 region into the adenovirus to be propagated.

The critical E4 region in the instant invention comprises, in the minimum, nucleic acid sequence encoding E4 ORF6 and can comprise the entire region of E4, inclusive of the promoter region. An important characteristic of the imported E4 region is that it is native to a virus of the same or highly similar serotype as the E1 gene product(s) (particularly E1B 55K) of the E1-complementing cell line, but heterologous to (i.e., non-native to a virus of the same serotype as) the adenoviral vector being propagated. As will be detailed below, the heterologous E4 region or portion thereof can be varied and can be inserted into the vector backbone at numerous locations.

The heterologous E4 region or portion thereof can, for instance, be a nucleic acid sequence encoding the entire open reading frame of the non-native E4. This segment of nucleic acid sequence can, in turn, be incorporated into the "native" entire E4 open reading frame of the recipient virus. In such an embodiment, the promoter native to the adenoviral vector would drive the expression of the non-native E4 region within the recombinant replication-defective adenoviral vector. Alternatively, the nucleic acid sequence encoding the entire open reading frame can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

In another embodiment, the heterologous E4 region comprises a nucleic acid sequence encoding the entire open reading frame of E4 and includes a non-native E4 promoter. In this type of embodiment, the E4 region can be inserted into the location of the combined native E4 and E4 promoter region. The non-native E4 region in this embodiment would be driven by expression of the non-native E4 promoter. Alternatively, the nucleic acid sequence encoding the entire open reading frame and the non-native E4 promoter can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

An alternative and further embodiment exists wherein the heterologous E4 region or portion thereof comprises nucleic acid sequence encoding a partial E4 region comprising ORF6 (one aspect of which is a region solely encoding ORF6). In this particular aspect of the invention, the heterologous non-native E4 protein can, in certain embodiments, replace the non-native ORF6 region or the entire E4-encoding region of the native virus. In the latter situation, the promoter driving expression of the non-native ORF6 can either be the native E4 promoter or a heterologous, non-native promoter operatively linked to the non-native ORF6, while in the latter, the expression of the non-native ORF6 would generally be driven by the native E4 promoter. Alternatively, the nucleic acid sequence encoding a partial E4 region comprising ORF 6 can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

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As one of skill in the art can appreciate, there are various ways in which one can envision the supply of a heterologous E4 nucleic acid sequence in cis to an adenoviral vector and thereby enable its growth based on Applicants' novel findings herein. Moreover, as one of skill in the art can appreciate, either native or non-native promoters can be utilized to drive expression of the heterologous E4 region or portion thereof.

Adenovirus pre-plasmids (plasmids comprising the genome of the replication-defective adenovirus with desired deletions and insertions) can be generated by homologous recombination using adenovirus backbones and an appropriate shuttle vector (designed to target-in specific deletions and incorporate desired restriction sites into the resultant plasmid). Shuttle vectors of use in this process can be generated using general methods widely understood and appreciated in the art, e.g., PCR of the adenoviral terminal ends taking into account the desired deletions, and the sequential cloning of the respective segments into an appropriate cloning plasmid. The adenoviral pre-plasmid can then be digested and transfected into the complementing cell line via calcium phosphate co-precipitation or other suitable means. Virus replication and amplification then occurs, a phenomenon made evident by notable cytopathic effect. Infected cells and media are then harvested after viral replication is complete (generally, 7-10 days post-transfection).

It is to be noted that various alternative adenoviral serotypes can be developed in accordance with the disclosed methods and, particularly, alternative adenoviral serotype vectors that were previously unable to be propagated or very inefficiently propagated utilizing existing adenoviral production cell lines based on subgroup C complementing E1 sequence. The various adenoviral vectors that can be developed in accordance with the instant methods include adenoviral vectors of subgroups A-F (for instance, serotypes of subgroups A, B (e.g., serotypes

11, 14, 16, 21, 34 and 35), C (e.g., serotypes 2 and 5), D (e.g., serotypes 24, 26 and 36), E (e.g., serotype 4) and F.

In preferred embodiments, the various non-group C family members can be developed with heterologous E4 supplied from a subgroup C member such as adenovirus serotype 5. Particular embodiments of the instant invention utilize a development scheme wherein the heterologous E4 protein is derived from a wildtype adenovirus serotype 5 sequence; see, e.g., a viral sequence which has been deposited with the American Type Culture Collection ("ATCC") under ATCC Deposit No. VR-5 (for which a transcription map can be found in Figure 1). A particular example of this type of embodiment is wherein an adenovirus of subgroup B (or any non-C subgroup) comprising heterologous E4 proteins in cis from Ad5 is propagated in Ad5 E1-complementing cell lines, for instance, PER.C6TM or 293. Applicants have, in fact, successfully propagated E1- serotypes 10, 24, 34, and 35 via use of this particular embodiment.

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One of skill in the art can readily identify alternative adenovirus serotypes (e.g., alternative serotypes of subgroups A, B (e.g., serotypes 11, 14, 16, 21, 34 and 35), C, (e.g., serotypes 2 and 5), D (e.g., serotypes 24, 26 and 36), E (e.g., serotype 4) and F) for the supply of the heterologous E4 protein. As long as the heterologous E4 region (or portion thereof comprising ORF6) of the vector is native to a virus of the same or highly similar serotype as the E1 region of the complementing cell line, the methods of the instant invention are widely applicable to the propagation and rescue of adenovirus of all serotypes. In light of the present disclosure, one can readily envision, for instance, how a complementing cell line based on a non-subgroup C adenovirus (e.g., the Ad35 cell line of EP 1 054 064 A1) can be utilized to propagate a virus of an adenoviral vector of subgroup C (e.g., adenovirus serotype 5) provided that the appropriate nucleic acid sequence encoding an E4 protein provided in cis is native to a virus of the same or highly similar serotype as that of the E1 expressed by the complementing cell line (i.e., an Ad35 E4 protein).

Complementing cell lines of use in the instant invention are available in the art and are not limited to any specific type. The critical feature, again, is that the heterologous segment of E4-encoding nucleic acid sequence provided in cis to the replication-defective vector being propagated be native to a virus of the same or highly similar serotype as the E1 expressed by the complementing cell line. One aspect of the instant invention employs E1-complementing cell lines wherein the expressed E1 is of serotype 5; e.g., PER.C6TM and 293 cell lines. Both these cell lines express the adenoviral E1 gene product. PER.C6TM is described in Fallaux et al., 1998 Human Gene Therapy 9:1909-1917, hereby incorporated by reference. 293 cell lines are described in Graham et al., 1977 J. Gen. Virol. 36:59-72, hereby incorporated by reference.

Another aspect of the instant invention are the adenoviral vectors of any serotype falling with adenoviral subgroups A, B, C, D, E and F (for instance, alternative serotypes of subgroups A, B (e.g., serotypes 11, 14, 16, 21, 34 and 35), C (e.g., serotype 2), D (e.g., serotypes 24, 26 and 36), E (e.g., serotype 4) and F) which are modified to contain a non-native E4-encoding nucleic acid sequence in cis which comprises, in whole or in part, nucleic acid sequence encoding open reading frame 6 (ORF6). Virus in accordance with this description can be propagated in accordance with the above-described methods and rescued using any suitable means known in the art.

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Another aspect of the instant invention is a vector in accordance with the instant invention which comprises a heterologous passenger gene in addition to that of the heterologous E4 nucleic acid sequence. In specific embodiments, the passenger gene encodes an antigen.

As one of ordinary skill in the art will appreciate, the instant methods are not limited by the heterologous gene that can be incorporated. The instant invention relates generally to a means by which to propagate multiple serotypes of adenovirus in a single complementing cell line and the recombinant virus that make the process possible. In preferred embodiments, the passenger gene is incorporated into the E1 deletion. In alternatively preferred embodiments, the passenger gene is inserted in an E3-deleted region. The position of the passenger gene, as one of ordinary skill in the art will appreciate, can be varied according to the specific complementing cell utilized and the specific deletions present within the replication-defective adenovirus genome.

In specific embodiments the passenger gene can encode an HIV-1 antigen, and in more preferred embodiments selected from the group consisting of genes encoding HIV-1 gag, pol, nef and env. In alternative embodiments, the passenger gene can be a reporter gene, such as secreted alkaline phosphatase (SEAP).

The passenger gene preferably exists in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid sequence encoding a protein of interest; (b) a promoter operatively linked to the nucleic acid sequence encoding the protein; and (c) a transcription termination sequence. The transcriptional promoter of the adenoviral vector is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al., 1991 Nucl. Acids Res. 19:3979-3986), which is hereby incorporated by reference), in certain embodiments without intronic sequences. Those skilled in the art, however, will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters

may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

The promoter may comprise a regulatable sequence such as the Tet operator sequence. This is extremely useful, for example, in cases where the gene products are affecting a result other than that desired and repression is sought.

Transcription termination sequences can also be utilized within the gene expression cassettes. Preferred termination sequences are, for instance, the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows:

10 AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTGTGTTTTTTTGTGTG (SEQ ID NO:4).

Further embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA.

The following non-limiting Examples are presented to better illustrate the

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EXAMPLE 1

Construction and Rescue

An E1- Ad35-based pre-adenovirus plasmid was constructed in order to determine whether an E1- Ad35 vector (a representative group B serotype) could be propagated in a group C E1-complementing cell line. The general strategy used to recover Ad35 as a bacterial plasmid is illustrated in Figure 3. Cotransformation of BJ5183 bacteria with purified wild-type Ad35 viral DNA and a second DNA fragment termed the Ad35 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome (see Figures 2A-1 to 2A-10) separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad5 457 to 3402 with a unique Swa I site located in the deletion. The Ad35 sequences in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which recombination can occur. The ITR cassette was also designed to contain unique restriction enzyme sites (Pme I) located at the end of the viral ITR's so that digestion will release the Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1. Pre-Adenovirus plasmid pAd35ΔE1 contains Ad35 sequences from 4 to 456 and bp 3403 to 34793.

To determine if pre-adenovirus plasmid pAd35ΔE1 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into a T-25 flask of PER.C6 cells using the calcium phosphate co-precipitation technique. *Pme* I digestion releases the viral genome from the plasmid sequences allowing viral replication to occur after entry into 293 cells. Viral cytopathic effect (CPE), indicating that virus replication and amplification is occurring, was never observed. Cells and media from the transfection were harvested at 14 days post transfection, freeze-thawed three times, clarified by centrifugation and used to infect new PER.C6 cells but no virus was ever amplified. Following multiple attempts, we have been unable to rescue and amplify pAd35ΔE1 in PER.C6 cells.

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EXAMPLE 2

Insertion of Ad5 Orf 6 and Ad5 E4 into the Ad5 Genome

To refine the strategy of including Ad5 Orf6 in the genome of an alternative serotype so that propagation could take place in a Ad5/group C complementing cell line four additional strategies were developed. In the first strategy, the entire alternative serotype E4 region (not including the E4 promoter) was deleted and replaced with Ad5 Orf6. In the second strategy, just the alternative serotype Orf6 gene was deleted and replaced with Ad5 Orf6. In the third strategy, the entire alternative serotype E4 coding region (not including the E4 promoter) was deleted and replaced with the Ad5 E4 coding region (not including the Ad5 E4 promoter) and, in the final strategy, the entire alternative serotype E4 coding and promoter region was deleted and replaced with the Ad5 E4 promoter and coding region. The configuration of the E4 regions generated by the four strategies is diagramed in Figure 4. For each of these strategies the desired pre-Adenovirus plasmid was generated by bacterial recombination. Cotransformation of BJ 5183 bacteria with purified wild-type viral DNA and the appropriately constructed ITR cassette resulted in the circularization of the viral genome by homologous recombination. The construction of each pre-Ad plasmid, based on Ad35, is outlined below:

To construct pAd35 Δ E1 Δ E4Ad5Orf6 (An Ad35 pre-Ad plasmid containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31913 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 31913 and 34419 generating pNEBAd35-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a

bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad35 bp 31912 to 34418 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct, Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31913 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria (Figure 5). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5Orf6. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5Orf6 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31913 and bp 34419 to bp 34793 with Ad5Orf6 cloned between bp 31913 and bp 34419.

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To construct pAd35ΔE1ΔOrf6Ad5Orf6 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 Orf6 substituted with Ad5 Orf6), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 32081 and bp 32990 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-10. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 32081 and 32990 generating pNEBAd35-10Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique Swa I restriction site located in the deletion and a deletion of E4 Orf6 from Ad35 bp 32082 to 32989 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct, Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 32081 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (Pme I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔOrf6Ad5Orf6. Pre-Adenovirus plasmid pAd35ΔE1ΔOrf6Ad5Orf6 contains Ad35

sequences from bp 4 to 456; bp 3403 to bp 32081 and bp 32990 to bp 34793 with Ad5Orf6 cloned between bp 32081 and bp 32990.

To construct pAd35ΔE1ΔE4Ad5E4 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 substituted with Ad5 E4), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31838 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-7. Next the Ad5 E4 coding region was generated by PCR and cloned between Ad35 bp 31838 and 34419 generating pNEBAd35-7Ad5E4-2 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad35 bp 31839 to 34418 into which the Ad5 E4 coding region was introduced in an E4 parallel orientation. In this construct, the Ad5 E4 region is expressed using the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31838 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (Pme I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5E4. Pre-Adenovirus plasmid pAd35AE1AE4Ad5E4 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31838 and bp 34419 to bp 34793 with the Ad5 E4 coding region (Ad 5 bp 32914 to bp 35523) cloned between bp 31838 and bp 34419.

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To construct pAd35\Delta E1\Delta E4Ad5PE4 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 coding region and promoter substituted with Ad5 E4 coding region and promoter), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31838 and bp 34660 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-8. Next the Ad5 E4 promoter and coding region was generated by PCR and cloned between Ad35 bp 31838 and 34660 generating pNEBAd35-8Ad5E4PC (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication,

ampicillin resistance gene, and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad35 bp 31839 to 34659 into which the Ad5 E4 promoter and coding region was introduced in an E4 parallel orientation. In this construct, the Ad5 E4 region is expressed using the Ad5 E4 promoter. The Ad35 sequences (bp 31599 to 31838 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (Pme I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5PE4. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5PE4 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31838 and bp 34660 to bp 34793 with the Ad5 E4 promoter and coding region (Ad 5 bp 32914 to bp 35826) cloned between bp 31838 and bp 34660.

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EXAMPLE 3 Rescue of pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4 into Virus

In order to determine if pre-adenovirus plasmids pAd35ΔE1ΔE4Ad5Orf6, pAd35 Δ E1 Δ Orf6Ad5Orf6, pAd35 Δ E1 Δ E4Ad5E4 and pAd35 Δ E1 Δ E4Ad5PE4 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmids were each digested with Pme I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique; Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc. PmeI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring, was observed for all construct. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect aT-225 flasks of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then

digested with HindIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with Pme1/HindIII prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 4

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Insertion of an Expression Cassette into pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4

In order to introduce a gag or SEAP expression cassette into the E1 region of the various Ad35 pre-Adenovirus plasmids described above (pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4) bacterial recombination was again used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence (Figure 6), was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2 (a precursor to the Ad35 ITR cassettes described above), generating pNEBAd35CMVgagBGHpA. pNEBAd35-2 contains Ad35 sequences from the left end of the genome (bp 4 to 456 and bp 3403 to 3886) with a unique SwaI site between bp 456 and 3403 at the position of the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pNEBAd35-2. This cloning step resulted in the gag expression cassette being cloned into the E1 deletion between bp 456 and 3403 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad35 bp 4 to 456 and bp 3403 to 3886 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad35 pre-Ad plasmids (pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4, pAd35ΔE1ΔE4Ad5PE4), linearized in the E1 region by digestion with Swa I, resulted in the generation of corresponding Ad35 gag-containing pre-Adenovirus plasmids (pAd35ΔE1gagΔE4Ad5Orf6, pAd35ΔE1gagΔOrf6Ad5Orf6, pAd35 Δ E1gag Δ E4Ad5E4, and pAd35 Δ E1gag Δ E4Ad5PE4) by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad35 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence (Figure 7) was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2, generating pNEBAd35CMVSEAPBGHpA. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pNEBAd35-2. The transgene was then recombined into the various Ad35 backbones generating pAd35ΔE1SEAPΔE4Ad5Orf6, pAd35ΔE1SEAPΔE4Ad5E4, and pAd35ΔE1SEAPΔE4Ad5PE4 as described above for the gag transgene. All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

15 EXAMPLE 5

In vivo Transgene Expression

A. Immunization

Female mice were between 4-10 weeks old. The total dose of each vaccine was
suspended in 0.1 mL of buffer. The vectors were given to both quadriceps of each animals with a volume of 50 μL per quad and using 0.3-mL 28G1/2 insulin syringes (Becton-Dickinson, Franklin Lakes, NJ). The rhesus macaques and African green monkeys were between 2-5 kg in weight. For the primates, the total dose of each vaccine was suspended in 1 mL of buffer. The monkeys were anesthetized (ketamine/xylazine mixture) and the vaccines were delivered i.m. in 0.5-mL aliquots into two muscle sites using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Serum samples were collected at defined intervals and stored frozen until the assay date. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National
Research Council.

B. SEAP Assay

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Serum samples were analyzed for circulating SEAP levels using TROPIX phospha-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 μ L aliquots of each serum were mixed with 45 μ L of kit-supplied dilution buffer in a 96-well white DYNEX plate.

Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey or mouse serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the well for 30 minutes at 65 °C. Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorder using DYNEX luminometer. RLU readings are converted to ng/mL SEAP using a log-log regression analyses.

C. Rodent Results

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In the first mouse experiment, cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^10 vp Ad35ΔE1SEAPΔE4Ad5Orf6; (2) 10^10 vp Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6; or (3) 10^10 vp Ad35ΔE1SEAP. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 8. Results indicate that (1) the Ad35 constructs are all capable of expressing the SEAP transgene and that (2) the introduction of Ad5Orf6 sequence where the deleted Ad35E4 was did not significantly affect the transgene expression relative to Ad35ΔE1SEAP. Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6 also yielded a similar expression profile as Ad35ΔE1SEAP. The levels of SEAP in the serum dropped after day 2 and were at background levels by day 12.

The second mouse experiment evaluates the effect of a full Ad5E4 replacement instead of an Ad5Orf6 substitution for the Ad35 E4 cassette. Here, cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^10 vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; (3) 10^10 vp Ad35ΔE1SEAPΔE4Ad5Orf6; (4) 10^10 vp Ad35ΔE1SEAPΔE4Ad5E4; or (5) 10^10 vp Ad35ΔE1SEAPΔE4Ad5PE4. The introduction of Ad5E4 or Ad5PE4 resulted in comparable if not, slightly improved expression levels compared to the vector with the Ad5Orf6 insertion (Figure 9). The peak levels for the Ad35 constructs are lower than those produced by Ad5SEAP (at least 10-fold).

D. Primate Results

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the following vectors: (1) 10^11 vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; or (3) 10^11 vp Ad35\Delta E1SEAP\Delta E4Ad5Orf6. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results for the individual monkeys are shown in Figures 10A-B. Results indicate that the peak level of SEAP product produced by the alternative adenovirus serotype was lower than but were within 3-fold of that of MRKAd5SEAP at the same

PCT/US2003/026145 WO 2004/018627

high dose level of 10^11 vp. The levels observed from the Ad35 vector were about 50-fold higher than those observed using 10^9 vp of MRKAd5SEAP. The levels of SEAP in the serum dropped after day 10 and were close to background as early as day 15.

A separate experiment using African green monkeys was conducted to examine the effect of the additional E3 deletion or the full Ad5E4 substitution on in vivo gene expression. In here, cohorts of 2-3 African green macaques were given single intramuscular injections of one of the following vectors: (1) 10^11 vp MRKAd5-SEAP; (2) 10^10 vp MRKAd5-SEAP; (3) 10^9 vp MRKAd5-SEAP; (4) 10^10 vp Ad35ΔE1SEAPΔE4Ad5Orf6; (5) 10^10 vp Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6; or (6) 10^10 vp Ad35ΔE1SEAPΔE4Ad5E4. Results (Figure 11) indicate that the peak levels of SEAP product produced by Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6 10 and Ad35ΔE1SEAPΔE4Ad5E4 were comparable if not, slightly improved compared to Ad35\DelseAP\Delate4Ad5Orf6.

EXAMPLE 6

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In vivo Immunogenicity 15

A. Immunization

Cohorts of 3-6 animals were given intramuscular injections at wk 0 and wk 4 of either of the following constructs: (1) 10^11 vp MRKAd5-HIV1 gag; or (2) 10^11 vp of Ad35ΔE1gagΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson). Sera and peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

B. ELISPOT Assay 30

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen et al., 2001 J. Virol. 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 µL of 2-4 x 10⁵ peripheral blood mononuclear cells (PBMCs)

were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 μL of media or the gag peptide pool at 8 μg/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO₂ for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10⁶ cell input.

C. Intracellular Cytokine Staining

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To 1 ml of 2 x 10⁶ PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μ g/mL. For gag-specific stimulation, 10 μ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO₂, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μL per tube antihCD3-APC, clone FN-18 (Biosource); 20 μL anti-hCD8-PerCP, clone SK1 (Becton Dickinson, Franklin Lakes, NJ); and 20 µL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μL 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4+ and CD8+ populations, and for both mock and gag-peptide reaction tubes of a sample.

30 D. Results

PBMCs collected at regular 4-wk intervals were analyzed in an ELISPOT assay. Results (Table 1) indicate that the Ad35ΔE1gagΔE4Ad5Orf6 is able to induce in non-human primates significant levels of gag-specific T cells. After a single dose (wk 4), the Ad35-induced responses were about 5-fold lower than that of MRKAd5-HIV1 gag. After the second dose (wk

8), the responses between both cohorts were comparable; the differences became pronounced in the succeeding time points.

Table 1. Gag-specific T cell response in monkeys immunized with MRKAd5-HIV1gag and Ad35ΔE1gagΔE4Ad5Orf6. Shown is the number of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag H peptide pool. The H pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

	Vaccine Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8		Wk 12		Wk 16	
Сгр			Mock	Gag H	Mock	Gag H	Mock	Gag H	Mock	Gag H	Mock	Gag H
1	MRKAd5-HIV1 gag 10^11 vp	00C018 00C034 00C058	1 0 4	5 4 4	13 5 3	1025 219 1086	0 5 0	824 404 440	3 0 0	.753 491 439	1 1 0	533 350 599
2	Ad35aE1gagaE4Ad5Ori6 10*11 vp	00D045 00D087 00D068 00D054 00D075 00D073	1 1 3 3 14	1 4 4 15 5 26	3 5 10 10 18 1	168 89 34 195 275 241	5 0 5 0 13 3	645 103 365 501 716 485	4 0 3 3 3 3	178 76 143 350 158 278	0 0 0	91 19 95 124 103 148
	Naïve	00D087	1	1	3	3	- 8	54	3	5	3	1_1

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Intracellular IFN-γ staining analyses of PBMC collected at wk 8 suggest that the Ad35-based vaccine is able to induce both HIV-specific CD4+ and CD8+ T cells (Table 2).

Table 2. Characterization of the gag-specific T cells in monkeys immunized with MRKAd5-HIV1gag and Ad35ΔE1gagΔE4Ad5Orf6. Shown are the percentages of CD3+ T cells that are either gag-specific CD4+ or gag-specific CD8+ cells. These values were corrected for mock values (<0.02%).

Grp	Vaccine	Monkey	Wk 8			
Gib	Wk 0, Wk 4	ID	%CD4+CD3+	%CD8+CD3+		
1	MRKAd5-HIV1 gag 10^11 vp	00C018 00C034 00C058	0.08 0.09 0.03	0.37 0.06 0.21		
2	Ad35∆E1gag∆E4Ad5Orf6 10^11 vp	00D045 00D067 00D068 00D054 00D075 00D073	0.06 0.02 0.15 0.05 0.08 0.09	0.08 0.02 0.02 0.08 0.05 0.06		

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In a separate experiment, 3 different Ad35 constructs expressing HIV-1 gag were evaluated for their immunogenicity in macaques. Here, cohorts of 3 macaques were given immunizations at wk 0 and 4 of either of the following vectors: (1) 10^10 vp Ad35ΔE1gagΔE4Ad5Orf6; (2) 10^10 - 22 -

vp Ad35ΔE1gagΔE3ΔE4Ad5Orf6; or (3) 10^10 vp Ad35ΔE1gagΔE4Ad5E4. The levels of T cell immunity induced by all 3 vectors were comparable at this stage (Table 2), suggesting that the additional E3 deletion or full Ad5E4 substitution does not appear to impair the immunogenic properties of the vector.

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Table 3. Gag-specific T cell response in monkeys immunized with several Ad35ΔE1ΔE4-based vectors. Shown is the number of spot-forming cells per million PBMC following incubation in the absence (mocK0 or presence of Gag H peptide pool. The H pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

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Grp	Vaccine	Monkey	Pre		Wk 4		Wk 8	
Gip	Wk 0, Wk 4	ID	Mock	Gag H	Mock	Gag H	Mock	Gag H
	Ad35∆E1gag∆E4Ad5Orf6	00C047	4	1	0	20	0	189
' '	10^10vp	00C157	8	5	1	81	1	833
	ιο τουρ	00C078	3	1	0	46	4	349
	Ad35AE1gagAE3AE4Ad5Orf6	00C091	1	1	1	118	3	315
	10^10vp	00C122	3	0	0	31	1	138
	10 1046	00D177	3	3	1	45	1	64
	4.05154A54Ad554	00D018	-3	19	29	120	23	193
3	Ad35∆E1gag∆E4Ad5E4	00D046	8	5	1	21	10	143
	10^10vp	00D043	3	4	ò	63	4	371
					 	ND	0	0
Naîve	попе	00D363	0	5	ND _	ND		

EXAMPLE 7 Construction and Rescue of pAd24ΔE1.

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An E1- Ad24-based pre-adenovirus plasmid was constructed in order to determine whether an E1- Ad24 vector (a representative group D serotype) could be propagated in an Ad5/group C E1-complementing cell line. Since at the time the vector construction was initiated the complete sequence of Ad24 (see Figures 16A-1 through 16A-10; subject of copending application serial no. 60/455, 312, filed March 17, 2003) was unknown we took advantage of some sequence homology between Ad24 and Ad17. The general strategy used to recover Ad24 as a bacterial plasmid is illustrated in Figure 12 and described below. Cotransformation of BJ5183 bacteria with purified wild-type Ad24 viral DNA and a second DNA fragment termed the Ad17 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 34469 to 35098) and left (bp 4 to 414 and bp 3373 to 4580) end of the Ad17 genome (Accession No. AF108105) separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad17

(bp 415 to 3372) with a unique Swa I site located in the deletion. The Ad17 sequences in the ITR cassette provide regions of homology with the purified Ad24 viral DNA in which recombination can occur. The ITR cassette was also designed to contain unique restriction enzyme sites (Pme I) located at the end of the viral ITR's so that digestion will release the Ad24 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd24ΔE1. pAd24ΔE1 contains Ad17 sequences from bp 4 to 414 and from bp 3373 to 4580, Ad24 bp 4588 to 34529, and Ad17 bp 34469 to 35098 (bp numbers refer to the wt sequence for both Ad17 and Ad24). PAd24ΔE1 contains the coding sequences for all Ad24 virion structural proteins that constitute its serotype specificity. This approach can be used to circularize any group D serotype into plasmid form which has sufficient homology to Ad17.

To determine if pre-adenovirus plasmid pAd24ΔE1 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into a 6 cm dish of 293 cells using the calcium phosphate co-precipitation technique. *Pme* I digestion releases the viral genome from the plasmid sequences allowing viral replication to occur after entry into 293 cells. Viral cytopathic effect (CPE), indicating that virus replication and amplification is occurring, was very slow to arise. Following multiple attempts, we were successful at rescuing and amplifying Ad24ΔE1 but the virus grew to lower titers and took more passages to amplify than a similar Ad5 based vector. In order to verify the genetic structure of the virus, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hind*III and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pme*1/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

EXAMPLE 8

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Insertion of Ad5 Orf 6 into the E1 region of Ad24

In order to determine if the insertion of Ad5 E4 Orf6 into the Ad24 genome would allow more efficient propagation in a group C E1 complementing cell line we constructed an Ad24 based pre-adenovirus plasmid containing Ad5 Orf6 in the E1 region. In order to introduce Ad5 Orf6 in to the E1 region of pAd24ΔE1, bacterial recombination was used. An Ad5 Orf6 transgene consisting of the Ad5 Orf6 coding region flanked by the HCMV promoter and pA was cloned into the E1 deletion in an Ad17 shuttle vector (a precursor to the Ad17 ITR cassette). The Ad5 Orf6 transgene was cloned between bp 414 and 3373 in the E1 anti-parallel

orientation. The shuttle vector containing the Ad5 Orf6 transgene was digested to generate a DNA fragment consisting of the transgene flanked by Ad17 sequences (bp 4 to 414 and bp 3373 to 4580) and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pAd24ΔE1, which had been linearized in the E1 region by digestion with *Swa*I, resulted in the generation of pAd24ΔE1Ad5Orf6 by homologous recombination (Figure 13). Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1Ad5Orf6.

In order to determine if pre-adenovirus plasmid pAd24\Delta E1Ad5Orf6 could be rescued into virus and propagated in an Ad5/group C E1 complementing cell line, pAd24ΔE1Ad5Orf6 was digested with Pme I and transfected into a 6 cm dish of 293 cells using the calcium phosphate co-precipitation technique. PmeI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into 293 cells. Once complete viral cytopathic effect (CPE) was observed at approximately 7-10 days post transfection, the infected cells and media were freeze/thawed three times and the cell debris pelleted. The virus was amplified in two additional passages in 293 cells and then purified from the final infection by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the virus, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with HindIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The endlabeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pme1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

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EXAMPLE 9

Insertion of Ad5 Orf 6 into the E4 region of Ad24

To refine the strategy of including Ad5 Orf6 in the genome of an alternative serotype so that propagation could take place in an Ad5/group C complementing cell line two additional strategies were developed. In the first strategy, the entire alternative serotype E4 region (not including the E4 promoter) was deleted and replaced with Ad5 Orf6. In the second strategy, just the alternative serotype Orf6 gene was deleted and replaced with Ad5 Orf6. The configuration of the E4 regions generated by the two strategies is diagramed in Figure 14. For each of these strategies the desired pre-Adenovirus plasmid was generated by bacterial recombination. Cotransformation of BJ 5183 bacteria with pAd24ΔOrf6BstZ17I and the

appropriately constructed Ad24 E4 shuttle plasmid resulted in the generation of the desired Ad24 based pre-Ad plasmid. PAd24ΔOrf6BstZ17I, a derivative of pAd24ΔE1, was constructed so that the E4 region in the Ad24 pre-Ad plasmid could be easily modified using bacterial recombination. PAd24ΔOrf6BstZ17I contains a deletion in the E4 region from Ad24 bp 32373 to bp 33328 with a unique *BstZ*17I site located at the position of the deletion. The complete sequence of pAd24ΔOrf6BstZ17I consists of Ad17 sequences from bp 4 to 414 and from bp 3373 to 4580, Ad24 bp 4588 to 32372 and from 33329 to 34529, and Ad17 bp 34469 to 35098 (bp numbers refer to the wt sequence for both Ad17 and Ad24).

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To construct pAd24ΔE1ΔE4Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1 deletion and a deletion of E4 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed by digesting pAd24ΔE1 with *PmeI* and *BsrGI* and cloning the restriction fragment representing the E4 region (bp 31559 to bp 35164) into pNEB193, generating pNEBAd24E4. PNEBAd24E4 was then digested with *AccI* and *Eco*NI to remove the E4 coding sequences and ligated with an oligo designed to contain *BglII* and *XhoI* sites (underlined) (5'

- 15 ACTCGAGATGTATAGATCT (SEQ ID NO: 6); 5' CTAGATCTATACATCTCGAG (SEQ ID NO: 7)), generating pNEBAd24ΔE4. PNEBAd24ΔE4 was then digested with BgIII and XhoI and ligated with the Ad5 Orf6 gene, which was PCR amplified, generating pNEBAd24ΔE4Ad5Orf6. The PCR primers used to amplify the Ad5 Orf6 gene (5' GCACAGATCTTTGCTTCAGGAATATG (SEQ ID NO: 8); 5'
- GAGAACTCGAGGCCTACATGGGGGTAGAG (SEQ ID NO: 9)) were designed to contain BglII and XhoI sites (underlined above) for ligation with the pNEBAd24DE4 fragment. In the final step pNEBAd24ΔE4Ad5Orf6 E4 shuttle plasmid was digested with PvuI and PmeI, the restriction fragments were size fractionated by agarose gel electrophoresis and the desired fragment containing Ad5Orf6 flanked by Ad24 sequences was gel purified. Cotransformation of BJ 5183 bacteria with E4 shuttle fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with BstZ17I, resulted in the generation of pAd24ΔE1ΔE4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1ΔE4Ad5Orf6.

To construct pAd24ΔE1ΔOrf6Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1 deletion and a deletion of E4 Orf6 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed in which the Ad24 Orf6 gene was replaced by Ad5 Orf6. To do this the *Eco*R1 restriction fragment representing bp 32126 to bp 33442 of the Ad24 genome (encompassing the E4 Orf6 coding region), was subcloned into the *Eco*RI site in pNEB193, generating pNEBAd24Orf6. In order to delete the E4 Orf6 gene in pNEBAd24Orf6 and replace it with Ad5 Orf6, pNEBAd24Orf6 was digested with *Sty*I and treated with Klenow to blunt the ends and then

digested with to EagI. The desired pNEBAd24Orf6 fragment was then ligated with a PCR product representing the Ad5 Orf6 gene from Ad5 bp 33193 to bp 24125, generating pNEBAd24ΔOrf6Ad5Orf6. The PCR primers used to generate the Ad5 Orf6 fragment (5'CGAGACGCCGACGCAGATCTGTTTG (SEQ ID NO: 10);

5 'GAAGTCCCGGGCTACATGGGGGTAG (SEQ ID NO: 11)) were designed to contain EagI and SmaI sites (underlined above) for ligation with the pNEBAd24Orf6 fragment. In the final step pNEBAd24ΔOrf6Ad5Orf6 was digested with EcoRI, the restriction fragments were size fractionated by agarose gel electrophoresis and the desired fragment containing Ad5Orf6 flanked by Ad24 sequences was gel purified. Cotransformation of BJ 5183 bacteria with the EcoRI fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with BstZ17I, resulted in the generation of pAd24ΔE1ΔOrf6Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as preadenovirus plasmid pAd24ΔE1ΔOrf6Ad5Orf6.

EXAMPLE 10

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Rescue of pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6, into Virus

In order to determine if pre-adenovirus plasmids pAd24ΔE1ΔE4Ad5Orf6, pAd24AE1AOrf6Ad5Orf6, could be rescued into virus and propagated in a group CE1 complementing cell line, the plasmids were each digested with Pme I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique; (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PmeI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring, was observed for both constructs. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect T-225 flasks of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with HindIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared

with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with Pme1/HindIII prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 11 5

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Comparison of the Growth Kinetics of Ad24 based vectors.

In order to compare the growth kinetic of Ad24ΔE1, Ad24ΔE1Ad5Orf6, Ad24ΔE1ΔE4Ad5Orf6 and Ad24ΔE1ΔOrf6Ad5Orf6 one step growth curves were preformed (Figure 15). PER.C6 cells in 60 mm dishes were infected at 1 vp per cell with either Ad24ΔE1, Ad24ΔE1Ad5Orf6, Ad24ΔE1ΔE4Ad5Orf6 or Ad24ΔE1ΔOrf6Ad5Orf6. Cells and media were then harvested at various times post infection, freeze thawed three times and clarified by centrifugation. The amount of virus present in the samples was determined by quantitative PCR and is illustrated in Figure 15. This study demonstrates that Ad24 vectors that incorporate Ad5 Orf6 have a distinct growth advantage over Ad24ΔE1 in PER.C6 cells. The instant invention can be practiced with recombinant Ad24 vectors absent a heterologous Orf 6 region where the E1-complementing cell line expresses an Ad24 E1 region or, alternatively, E1 and E4 regions of the same serotype (such as Ad5E1/E4-expressing cell lines).

EXAMPLE 12

Insertion of an Expression Cassette into pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6, In order to introduce a gag or SEAP expression cassette (see Figures 6 and 7, respectively) into the E1 region of the Ad24 pre-Adenovirus plasmids described above (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6) bacterial recombination was used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, was cloned into the E1 deletion in Ad17 shuttle plasmid, pABSAd17-3, generating pABSAd17HCMVgagBGHpA. The ITR cassette contains sequences from the right (bp 34469 to 35098) and left (bp 4 to 414 and bp 3373 to 4580) end of the Ad17 genome separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad17 (bp 415 to 3372) with a unique Swa I site located in the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the Swal site in pABSAd17-3. This cloning step resulted in the gag expression cassette being 35

cloned into the E1 deletion between bp 414 and 3373 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad17 bp 4 to 414 and bp 3373 to 4580 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad24 pre-Ad plasmids (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6,), linearized in the E1 region by digestion with Swa I, resulted in the generation of the corresponding Ad24 gag-containing pre-Adenovirus plasmids (pAd24ΔE1gagΔE4Ad5Orf6, pAd24ΔE1gagΔOrf6Ad5Orf6) by homologous recombination. Potential clones were screened by restriction analysis.

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A similar strategy was used to generate Ad24 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence was cloned into the E1 deletion in Ad17 shuttle plasmid, pABSAd17-3, generating pABSAd17HCMVSEAPBGH. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pABSAd17-3. The shuttle vector containing the SEAP transgene was digested to generate a DNA fragment consisting of the SEAP expression cassette flanked by Ad17 bp 4 to 414 and bp 3373 to 4580 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad24 pre-Ad plasmids (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6,), linearized in the E1 region by digestion with Swa I, resulted in the generation of the corresponding Ad24 SEAP-containing pre-Adenovirus plasmids (pAd24ΔE1SEAPΔE4Ad5Orf6, pAd24ΔE1SEAPΔOrf6Ad5Orf6) by homologous recombination. Potential clones were screened by restriction analysis. All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described

EXAMPLE 13

above.

30 In Vivo Immunogenicity

A. Immunization

Cohorts of 3-6 animals were given intramuscular injections at wk 0 and wk 4 of either of the following constructs: (1) 10^11 vp MRKAd5-HIV1 gag; (2) 10^10 vp MRKAd5-HIV1 gag; (3) 10^11 vp of Ad24ΔE1gagΔOrf6Ad5Orf6; (4) 10^10 vp of

Ad24ΔE1gagΔOrf6Ad5Orf6; or (5) 10^10 vp of Ad24ΔE1gagΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points (typically 4 wk intervals) during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. ELISPOT Assay

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The IFN- γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen et al., 2001 *J. Virol.* 75(2):738-749; Casimiro et al., 2002 *J. Virol.* 76:185-94), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μ L of 2-4 x 10⁵ peripheral blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 μ L of media or the gag peptide pool at 8 μ g/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO₂ for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10⁶ cell input.

25 C. Intracellular Cytokine Staining

To 1 ml of 2 x 10⁶ PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μg/mL. For gag-specific stimulation, 10 μL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO₂, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μL anti-hCD8-PerCP, clone SK1 (Becton Dickinson);

and 20 μ L anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μ L 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μ g of FITC-anti-hIFN- γ , clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4⁺ and CD8⁺ populations, and for both mock and gag-peptide reaction tubes of a sample.

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D. Anti-p24 ELISA

A modified competitive anti-p24 assay was developed using reagents from the Coulter p24 Antigen Assay kit (Beckman Coulter, Fullerton, CA). Briefly, to a 250-µL serum sample, 20 µL of Lyse Buffer and 15 µL of p24 antigen (9.375 pg) from the Coulter kit were added. After mixing, 200 µL of each sample were added to wells coated with a mouse anti-p24 mAb from the Coulter kit and incubated for 1.5 hr at 37°C. The wells were then washed and 200 µL of Biotin Reagent (polyclonal anti-p24-biotin) from the Coulter kit was added to each well. After a 1 hr, 37°C incubation, detection was achieved using strepavidin-conjugated horseradish peroxidase and TMB substrate as described in the Coulter Kit. OD450nm values were recorded. A 7-point standard curve was generated using a serial 2-fold dilution of serum from an HIV-seropositive individual. The lower cut-off for the assay is arbitrarily set at 10 milli Merck units/mL (mMU/mL) defined by a dilution of the seropositive human serum. This cutoff falls at approximately 65% of the maximum bound control signal which corresponds to that obtained with the diluent control only and with no positive analyte.

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E. Results

PBMCs collected at regular 4-wk intervals were analyzed in an ELISPOT assay (Figure 17). Both Ad24ΔE1gagΔOrf6Ad5Orf6 and Ad24ΔE1gagΔE4Ad5Orf6 were able to induce significant levels of gag-specific T cells in non-human primates. At 10^11 vp dose level, the Ad24-induced responses were within 2-3-fold of those of MRKAd5-HIV1 gag. Both Ad24 vectors were also able to induce detectable levels of gag-specific T cells at 10^10 vp but were lower than those observed using MRKad5gag at the same dose.

PBMCs collected at wk 12 from the vaccinees were analyzed for intracellular IFN-γ staining after the priming immunizations. The assay results provided information on the relative amounts of CD4⁺ and CD8⁺ gag-specific T cells in the peripheral blood (Figure 18). The

results indicated that the prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4⁺ and CD8⁺ T cells.

F. Humoral Immune Responses

The Ad24-based vaccine vector was able to generate detectable levels of circulating anti-gag antibodies at the reasonably high dose level (Figure 19). No detectable titers were observed at equal to or lower than 10^10 vp, suggesting the existence of a dose-dependent response.

10 EXAMPLE 14

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In Vivo Transgene Expression

A. Immunization

Cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^10 vp Ad24ΔE1SEAPΔE4Ad5Orf6; (2) 10^10 vp Ad24ΔE1SEAPΔOrf6Ad5Orf6; (3) 10^10 vp MRKAd5SEAP; and (4) 10^9 vp MRKAd5SEAP. Female mice were between 4-10 weeks old. The total dose of each vaccine was suspended in 0.1 mL of buffer. The vectors were given to both quadriceps of each of the animals with a volume of 50 uL per quad and using 0.3-mL 28G1/2 insulin syringes (Becton-Dickinson, Franklin Lakes, NJ). For the primates, the total dose of each vaccine was suspended in 1 mL of buffer. The monkeys were anesthetized (ketamine/xylazine mixture) and the vaccines were delivered i.m. in 0.5-mL aliquots into two muscle sites using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Serum samples were collected at defined intervals and stored frozen until the assay date. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

Serum samples were analyzed for circulating SEAP levels using TROPIX phospha-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 uL aliquots of each serum were mixed with 45 uL of kit-supplied dilution buffer in a 96-well white DYNEX plate. Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the wells for 30 minutes at 65 °C.

Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorder using DYNEX luminometer. RLU readings are converted to ng/mL SEAP using a log-log regression analyses.

5 C. Rodent Results

Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 20. Results indicate that (1) both Ad24 constructs are all capable of expressing the SEAP transgene in vivo to comparable levels; and that (2) the level of expression achieved using the Ad24 vectors are comparable to that of Ad5 at 10-fold lower dose. The levels of SEAP in the serum dropped dramatically after day 2 and were at background levels by day 12.

D. Primate Results

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the following vectors: (1) 10^11 vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; (3) 10^11 vp Ad24ΔE1SEAPΔOrf6Ad5Orf6; or (4) 10^11 vp Ad24ΔE1SEAPΔE4Ad5Orf6. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 21.

Results indicate that the peak levels of SEAP product produced by adenovirus serotype 24 were lower than but were within 3-fold of that of MRKAd5 at the same high dose level of 10^11 vp (Figure 21). The levels observed with adenovirus serotype 24 are generally 50-fold higher than those observed using 10^9 vp of MRKAd5. The levels of SEAP in the serum dropped dramatically after day 10 and were close to background as early as day 15. These observations strongly indicate that adenovirus serotype 24 is very efficient in expressing a transgene following intramuscular administration in a primate.

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EXAMPLE 15

Construction of pMRKAd24\Delta E1\Delta E4Ad5Orf6

To construct pMRKAd24 Δ E1 Δ E4Ad5Orf6 (An Ad24 pre-Ad plasmid, composed entirely of Ad24 sequence and containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), an Ad24 ITR cassette was constructed containing sequences from the right (bp 31978 to 32264 and bp 34713 to 35164) and left (bp 4 to 450 and bp 3364 to 3799) end of the Ad24 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd24-4. Next the Ad5 Orf6 open reading frame (Ad5 bp 31192 to bp 34078) was generated by PCR and cloned between Ad24 bp 32264 and 34713 generating

pNEBAd24E-Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad24 bp 451 to 3363 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad24 bp 32265 to 34712 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5 Orf6 expression is driven by the Ad24 E4 promoter. The Ad24 sequences (bp 31978 to 32264 and bp 3464 to 3799) in the ITR cassette provide regions of homology with the purified Ad24 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria (Figure 22). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion will release the recombinant Ad24 genome from plasmid sequences. Potential clones will be screened by restriction analysis and one clone was selected as pMRKAd24ΔE1ΔE4Ad5Orf6. Pre-Adenovirus plasmid pMRKAd24\Delta E1\Delta E4Ad5Orf6 should contain Ad24 sequences from bp 4 to 450; bp 3364 to bp 32264 and bp 34713 to bp 35164 with Ad5Orf6 cloned between bp 32264 and bp 34713. The bp numbering in the above description refers to the wt sequence for both Ad24 and Ad5.

EXAMPLE 16

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Insertion of HIV-1 gag and SEAP transgenes into pAd24ΔE1ΔE4Ad5Orf6

In order to introduce a gag or SEAP expression cassettes into the E1 region of pMRKAd24ΔE1ΔE4Ad5Orf6, bacterial recombination will be used. An HIV-1 gag expression cassette will consist of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, in the E1 deletion of an Ad24 shuttle plasmid, pNEBAd24-2 (a precursor to the Ad24 ITR cassette described above), generating pNEBAd24CMVgagBGHpA. PNEBAd24-2 contains Ad24 sequences from the left end of the genome (bp 4 to 450 and bp 3364 to 3799) that define the E1 deletion. The gag expression cassette will be obtained from a previously constructed plasmid and cloned into the E1 deletion between bp 450 and 3364 in the E1 parallel orientation. The shuttle vector containing the gag transgene will be digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad24 bp 4 to 450 and bp 3364 to 3799 and the fragment will be purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pMRKAd24ΔE1ΔE4Ad5Orf6 which was linearized in the E1 region by digestion with SwaI, should result in the generation of Ad24 gag-

containing pre-Adenovirus plasmids pMRKAd24ΔE1gagΔE4Ad5Orf6 by homologous recombination. Potential clones will be screened by restriction analysis.

A similar strategy will be used to generate Ad24 pre-Ad plasmids containing a SEAP expression cassette. In this case, a SEAP expression cassette will consist of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence cloned into the E1 deletion of an Ad24 shuttle plasmid, pNEBAd24-2, generating pNEBAd24CMVSEAPBGHpA. The transgene will then be recombined into pMRKAd24 Δ E1 Δ E4Ad5Orf6 as described above for the gag transgene.

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EXAMPLE 17 In Vivo Immunogenicity

A. Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

25 B. T Cell Responses

Ad24 Vaccine Vector as a Heterologous Booster: Cohort of 4 rhesus macaques was immunized initially with 3 doses (wk 0, 4, 26) of either 10⁷ or 10⁹ vp of MRKAd5-gag (see, PCT/US01/28861, published March 21, 2002) or MRKAd6-gag. At wk 56, the animals received a booster vaccine of 10¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6. A separate cohort of naïve animals received a single dose of the booster vaccine. The results of the IFN-γ ELISPOT analyses of PBMC collected during the course of the studies are shown in Figure 23. It is apparent that the Ad24 HIV vectors can be utilized to amplify the existing pools of HIV-specific T cells. The increases in the levels of gag-specific T cells from the pre-boost levels to those measured at 4 wks post boost were consistently larger than the levels induced by the same booster vaccine in naïve animals. PBMCs from the vaccinees of the heterologous MRKAd5/MRKAd6-Ad24 boost

regimen were analyzed for intracellular IFN-γ staining after the priming immunizations (wk 60). The assay results provided information on the relative amounts of CD4⁺ and CD8⁺ gag-specific T cells in the peripheral blood (Figure 24). The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4+ and CD8+ T cells.

Ad24 Vaccine Vector as a Heterologous Primer: In a separate study, a cohort of 3 rhesus macaques was immunized initially with 2 doses (wk 0, 4) of 10¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6 and boosted at wk 24 with 10⁷ vp of MRKAd5-gag. The low dose of MRKAd5-gag is selected to mimic the effect of pre-existing neutralizing immunity to the vector in a subject. A separate cohort of naïve animals was given a single dose of 10⁷ vp MRKAd5-gag. The results of the IFN-γ ELISPOT analyses of PBMC collected during the course of the studies are shown in Figure 25.

The Ad24-based vaccine was able to prime effectively for HIV-specific T cell responses in macaques. Boosting with a low dose MRKAd5-gag resulted in a significant increase in the levels of gag-specific T cells. The increases in 2 out of 3 animals exceed the levels typically observed after treatment of naïve animals with the same low dose of MRKAd5-gag.

EXAMPLE 18

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20 Construction of pAd34ΔE1ΔE4Ad5Orf6

To generate an E1- Ad34 based vector that can propagate in existing group C/Ad5 E1 complementing cell lines (293, PER.C6), Ad5 Orf6 was inserted in place of the native E4 region. Since at the time, the complete sequence of Ad34 (see Figures 28A-1 to 28A-9; subject of copending application serial no. 60/458,825, filed March 28, 2003) was unknown, advantage was taken of the sequence homology between Ad34 and Ad35 in order to construct the Ad34 pre-Adenovirus plasmid. Cotransformation of BJ 5183 bacteria with purified wild-type Ad34 viral DNA and the appropriately constructed Ad35 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The construction of the pre-Ad plasmid based on Ad34, is outlined below:

To construct pAd34 Δ E1 Δ E4Ad5Orf6 (An Ad34 pre-Ad plasmid containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), we utilized an Ad35 ITR cassette. We anticipated that sequence homology between Ad34 and Ad35 would allow homologous recombination to occur. The Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31913 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome (see Figures 2A-1 to 2A-10) separated by plasmid sequences containing a

bacterial origin of replication and an ampicillin resistance gene. The four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 31913 and 34419 generating pNEBAd35-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad35 bp 31914 to 34418 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31913 and bp 3403 to 3886) in the ITR cassette provided regions of homology with the purified Ad34 viral DNA in which bacterial recombination could occur following cotransformation into BJ 5183 bacteria (Figure 26). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion would release the recombinant Ad34 genome from the plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd34 Δ E1 Δ E4Ad5Orf6.

EXAMPLE 19

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20 Rescue of pAd34ΔE1ΔE4Ad5Orf6 into Virus

In order to determine if pre-adenovirus plasmid pAd34AE1AE4Ad5Orf6, could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc). *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring was observed following transfection. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation.

Approximately 1 ml of the cell lysate was used to infect a T-225 flask of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE, the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment

followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *HindIII* and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme1/HindIII* prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 20

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Insertion of an Expression Cassette into pAd34ΔE1ΔE4Ad5Orf6

In order to introduce a gag or SEAP expression cassette (see Figures 6 and 7, respectively) into the E1 region of pAd34ΔE1ΔE4Ad5Orf6, bacterial recombination was again used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2 (a precursor to the Ad35 ITR cassettes described above), generating pNEBAd35CMVgagBGHpA. pNEBAd35-2 contains Ad35 sequences from the left end of the genome (bp 4 to 456 and bp 3403 to 3886) with a unique SwaI site between bp 456 and 3403 at the position of the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pNEBAd35-2. This cloning step resulted in the gag expression cassette being inserted into the E1 deletion between bp 456 and 3403 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad35 bp 4 to 456 and bp 3403 to 3886 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pAd34\Delta E1\Delta E4Ad5Orf6, linearized in the E1 region by digestion with Swa I, resulted in the generation of the Ad34 gag-containing pre-Adenovirus plasmid pAd34ΔE1gagΔE4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad34 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence was

cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2, generating pNEBAd35CMVSEAPBGHpA. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pNEBAd35-2. The transgene was then recombined into the pAd34ΔE1ΔE4Ad5Orf6, generating pAd34ΔE1SEAPΔE4Ad5Orf6 as described above for the gag transgene.

All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

10 EXAMPLE 21

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Construction of pMRKAd34ΔE1ΔE4Ad5Orf6

To construct an Ad34 pre-Ad plasmid that was composed entirely of Ad34 sequences, an Ad34 ITR cassette was generated. The Ad34 ITR cassette was constructed containing sequences from the right (bp 31584 to 31895 and bp 34409 to 34772) and left (bp 4 to 456 and bp 3402 to 3885) end of the Ad34 genome (see Figures 28A-1 to 28A-9) separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd34-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad34 bp 31895 and 34409 generating pNEBAd34-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad34 bp 457 to 3401 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad34 bp 31896 to 34408 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5Orf6 expression is driven by the Ad34 E4 promoter. The Ad34 sequences (bp 31584 to 31895 and bp 3402 to 3885) in the ITR cassette provided regions of homology with the purified Ad34 viral DNA in which bacterial recombination could occur following cotransformation into BJ 5183 bacteria (Figure 27). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion would release the recombinant Ad34 genome from the plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pMRKAd34 Δ E1 Δ E4Ad5Orf6.

EXAMPLE 22 In Vivo Studies

A. Immunization

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Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the two vectors: (1) 10^11 vp MRKAd5-SEAP (in MRKAd vector backbone disclosed in PCT/US01/28861, published March 21, 2002); and (2) 10^11 vp Ad34ΔE1SEAPΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

Serum samples were analyzed for circulating human secreted alkaline phosphatase (SEAP) levels using TROPIX phospha-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 μ L aliquots of each serum were mixed with 45 μ L of kit-supplied dilution buffer in a 96-well white DYNEX plate. Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the well for 30 minutes at 65 °C. Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorded using DYNEX luminometer. RLU readings were converted to ng/mL SEAP using a log-log regression analyses.

C. ELISPOT Assay

The IFN- γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μ L of 2-4 x 10⁵ peripheral blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower

size cut-off set at 80 femtoliters ("fL"). Either 50 μ L of media or the gag peptide pool at 8 μ g/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO₂ for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10^6 cell input.

D. Intracellular Cytokine Staining (ICS)

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To 1 ml of 2 x 106 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μ g/mL. For gag-specific stimulation, 10 μ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO₂, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 µL per tube antihCD3-APC, clone FN-18 (Biosource); 20 µL anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 µL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 µL 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4⁺ and CD8⁺ populations, and for both mock and gag-peptide reaction tubes of a sample.

E. Results

Expression: Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 29. Results indicate that the peak levels of SEAP protein produced by the alternative adenovirus serotype were lower than but were within 3-fold of that of MRKAd5 at the same high dose level of 10^11 vp (Figure 29). The levels of SEAP in the serum dropped dramatically after day 10 and were close to background as early as day 15. These observations strongly indicate that the Ad34-based vector is efficient in expressing a transgene following intramuscular administration in a primate.

Immunogenicity: Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 30; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool or the mock (no peptide) control.

Immunization with gag-expressing Ad34 vector induced detectable levels of circulating gag-specific T cells immediately after a single dose of the vector. The responses improved following a second dose given at wk 4. Overall, the responses to the Ad34-based vector were slightly lower than those induced by the same dose of MRKAd5-gag. The results strongly indicate the Ad34-based vector can prime effectively for HIV-specific T cell responses.

IFN-γ ICS analyses of the PBMC from the Ad34-immunized animals revealed that the vector can induce detectable levels of both CD4⁺ and CD8⁺ HIV-specific T cells (Figure 31).

15 EXAMPLE 23

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Heterologous Immunization

Cohorts of 3 monkeys were immunized (at wks 0, 4) with 10^11 vp Ad34\Delta E1gag\Delta E4Ad5Orf6 followed by a booster at week 24 with 10^10 vp Ad35\Delta E1gag\Delta E4Ad5Orf6. Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 32; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool or the mock (no peptide) control.

Immunization with gag-expressing Ad34 vector induced detectable levels of circulating gag-specific T cells that decreased to between 94-139 SFC/10^6 PBMC at the time of the boost. Heterologous immunization with an Ad35-based HIV vector resulted in as much as a 3-fold increase in T cell responses.

IFN- γ ICS analyses of the PBMCs from the Ad34 primed/Ad35 boosted animals at week 28 revealed that the vector can induce detectable levels of both CD4⁺ and CD8⁺ HIV-specific T cells (Figure 33).

WHAT IS CLAIMED IS:

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1. A means for propagating replication-defective adenovirus in an adenoviral E1-complementing cell line expressing E1 gene product(s) which are non-native to the adenovirus, which comprises:

- (a) inserting all or a portion of a heterologous adenoviral E4 region comprising nucleic acid sequence encoding open reading frame 6 (ORF6) into a replication-defective adenovirus; wherein the E4 region or portion thereof inserted into the adenovirus is native to a virus of the same adenovirus serotype as the E1 gene product(s) expressed by the complementing cell line;
- (b) introducing the replication-defective adenovirus into the adenoviral E1-complementing cell line;
- (c) allowing the replication-defective adenovirus to propagate in the adenoviral E1-complementing cell line; and
 - (d) rescuing the propagated adenovirus.
- 2. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof comprises the complete adenoviral E4-encoding region.
- 3. A means in accordance with claim 2 wherein the heterologous adenoviral E4 region or portion thereof comprises the complete adenoviral E4-encoding region and native E4 promoter.
- 4. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is inserted into the replication-defective virus in place of nucleic acid sequence encoding open reading frame 6 (ORF6).

5. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is inserted into the replication-defective virus in place of nucleic acid sequence encoding the complete adenoviral E4-encoding region.

- 6. A means in accordance with claim 1 wherein the heterologous adenoviral
 5 E4 region or portion thereof is derived from a subgroup C adenovirus.
 - 7. A means in accordance with claim 1 wherein the subgroup C adenovirus is adenovirus of serotype 5.
 - 8. A means in accordance with claim 7 wherein the replication-defective adenovirus is an adenovirus of subgroup B.
 - 9. A means in accordance with claim 7 wherein the replication-defective adenovirus is an adenovirus of serotype 35.

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- 10. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is operatively linked to a heterologous promoter.
- 11. A means in accordance with claim 1 wherein the adenoviral E1-complementing cell line is a PER.C6® cell line.
 - 12. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6).
 - 13. A replication-defective adenovirus in accordance with claim 12 wherein the adenovirus comprises a heterologous gene of interest.
 - 14. A replication-defective adenovirus in accordance with claim 13 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.
 - 15. A replication-defective adenovirus in accordance with claim 14 wherein the HIV-1 antigen is selected from the group consisting of HIV-1 gag, pol, nef and env.

16. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6) and a gene encoding HIV-1 gag.

- 17. A replication-defective adenovirus comprising all or a portion of a

 heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6) in
 place of a native E4 region or portion thereof comprising ORF6.
 - 18. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a complete heterologous E4 region in place of a complete native E4 region.
 - 19. A replication-defective adenovirus comprising a heterologous E4 region or portion thereof comprising a complete heterologous E4 region including E4 promoter in place of a complete native E4 region.

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- 20. Adenovirus propagated in accordance with the means of claim 1.
- 21. A means in accordance with claim 1 wherein the replication-defective adenovirus comprises a heterologous gene of interest.
 - 22. A means in accordance with claim 21 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.
 - 23. A means in accordance with claim 22 wherein the HIV-1 antigen is selected from the group consisting of: HIV-1 gag, pol, nef and env.
- 24. A replication-defective adenovirus of serotype 35 comprising all or a portion of an adenovirus serotype 5 E4 region comprising open reading frame 6 (ORF6) and a heterologous gene of interest.
 - 25. A replication-defective adenovirus in accordance with claim 24 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

26. A replication-defective adenovirus in accordance with claim 25 wherein the HIV-1 antigen is selected from the group consisting of: HIV-1 gag, pol, nef and env.

27. A replication-defective adenovirus of serotype 35 comprising all or a portion of an adenovirus serotype 5 E4 region comprising open reading frame 6 (ORF6) and a gene encoding HIV-1 gag.

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- 28. A recombinant adenoviral vector of serotype 24 which comprises an E4 gene or a segment of an E4 gene comprising open reading frame 6 ("ORF6") of an alternative serotype.
- 29. A population of cells comprising the recombinant adenoviral vector of claim 28.
 - 30. A method for producing recombinant, replication-defective adenovirus particles comprising:
 - (a) introducing a recombinant adenoviral vector of claim 28 into a population of cells expressing adenovirus E1; and
 - (b) harvesting the resultant recombinant, replication-defective adenovirus.
 - 31. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 30.
 - 32. A composition comprising purified recombinant adenovirus particles in accordance with claim 31.
 - 33. A composition in accordance with claim 32 which comprises a physiologically acceptable carrier.
 - 34. A recombinant adenoviral vector in accordance with claim 28 which is at least partially deleted in E1 and devoid of E1 activity and comprises a heterologous nucleic acid.

35. A composition comprising purified recombinant adenoviral particles in accordance with claim 31 which are at least partially deleted in E1 and devoid of E1 activity and comprise a heterologous nucleic acid.

36. A method for effecting the delivery and expression of heterologous nucleic acid comprising administering the composition of claim 35 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

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- 37. A method in accordance with claim 36 wherein the composition is preceded or followed by administration of heterologous nucleic acid with an adenovirus of a different serotype.
- 38. A composition in accordance with claim 35 wherein the heterologous nucleic acid encodes an HIV antigen.
- 39. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 38.
- 40. A composition in accordance with claim 39 wherein the HIV antigen is

 HIV-1 gag or immunologically relevant modification thereof.
 - 41. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.
 - 42. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.
 - 43. A recombinant adenoviral vector of serotype 24 which is at least partially deleted in E1 and devoid of E1 activity; wherein said vector comprises an E4 gene or a segment of an E4 gene from adenovirus serotype 5 comprising open reading frame 6 ("ORF6"), and a heterologous nucleic acid.

44. A population of cells comprising the recombinant adenoviral vector of claim 43.

45. A method for producing recombinant, replication-defective adenovirus particles comprising:

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- (a) introducing a recombinant adenoviral vector of claim 43 into a population of cells expressing adenovirus serotype 5 E1; and
 - (b) harvesting the resultant recombinant, replication-defective adenovirus.
- 46. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 45.
- 47. A composition comprising purified recombinant adenovirus particles in accordance with claim 46.
- 48. A composition in accordance with claim 47 which comprises a physiologically acceptable carrier.
- 49. A method for effecting the delivery and expression of the heterologous nucleic acid comprising administering the composition of claim 48 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.
- 50. A method in accordance with claim 49 above wherein the composition is preceded or followed by administration of the heterologous nucleic acid with an adenovirus of a different serotype.
- 51. A composition in accordance with claim 48 wherein the heterologous nucleic acid encodes an HIV antigen.
- 52. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 51.

53. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

- 54. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.
- 55. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

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- A recombinant adenoviral vector of serotype 34 which comprises an E4 gene or a segment of an E4 gene comprising open reading frame 6 ("ORF6") of an alternative serotype.
- 57. A population of cells comprising the recombinant adenoviral vector of claim 56.
- 58. A method for producing recombinant, replication-defective adenovirus particles comprising:
- (a) introducing a recombinant adenoviral vector of claim 56 into a population of cells expressing adenovirus E1; and
 - (b) harvesting the resultant recombinant, replication-defective adenovirus.
 - 59. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 58.
- 60. A composition comprising purified recombinant adenovirus particles in accordance with claim 59.
 - 61. A composition in accordance with claim 60 which comprises a physiologically acceptable carrier.
 - 62. A recombinant adenoviral vector in accordance with claim 56 which is at least partially deleted in E1 and devoid of E1 activity and comprises a heterologous nucleic acid.

63. A composition comprising purified recombinant adenoviral particles in accordance with claim 59 which are at least partially deleted in E1 and devoid of E1 activity and comprise a heterologous nucleic acid.

64. A method for effecting the delivery and expression of heterologous nucleic acid comprising administering the composition of claim 63 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

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- 65. A method in accordance with claim 64 wherein the composition is preceded or followed by administration of heterologous nucleic acid with an adenovirus of a different serotype.
- 66. A composition in accordance with claim 63 wherein the heterologous nucleic acid encodes an HIV antigen.
- 67. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 66.
- 68. A composition in accordance with claim 67 wherein the HIV antigen is
 HIV-1 gag or immunologically relevant modification thereof.
 - 69. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.
 - 70. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.
 - 71. A recombinant adenoviral vector of serotype 34 which is at least partially deleted in E1 and devoid of E1 activity; wherein said vector comprises an E4 gene or a segment of an E4 gene from adenovirus serotype 5 comprising open reading frame 6 ("ORF6"), and a heterologous nucleic acid.

72. A population of cells comprising the recombinant adenoviral vector of claim 71.

- 73. A method for producing recombinant, replication-defective adenovirus particles comprising:
- (a) introducing a recombinant adenoviral vector of claim 71 into a population of cells expressing adenovirus serotype 5 E1; and

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- (b) harvesting the resultant recombinant, replication-defective adenovirus.
- 74. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 73.
- 75. A composition comprising purified recombinant adenovirus particles in accordance with claim 74.
 - 76. A composition in accordance with claim 75 which comprises a physiologically acceptable carrier.
- 77. A method for effecting the delivery and expression of the heterologous nucleic acid comprising administering the composition of claim 76 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.
 - 78. A method in accordance with claim 77 above wherein the composition is preceded or followed by administration of the heterologous nucleic acid with an adenovirus of a different serotype.
- 79. A composition in accordance with claim 76 wherein the heterologous nucleic acid encodes an HIV antigen.
- 80. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 79.

81. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

- 82. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.
- 83. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

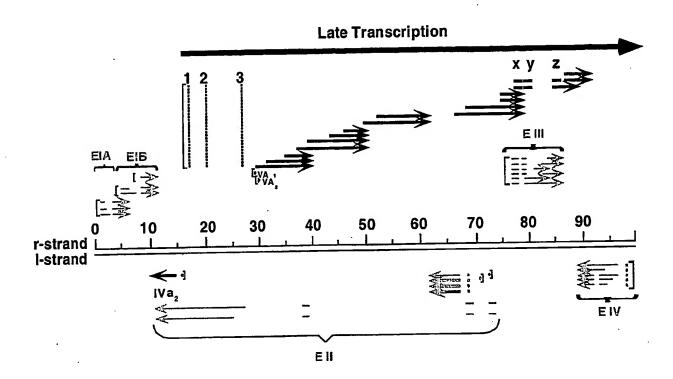


FIG. 1

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<i>CC</i> 1	agatagagag	ccacctatac	agetttttga	acctcctacg	cttcaggaac	Lycatyatti
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		. ~~~~~~++~~	tractition	aradiciolo	acaaaacaaa	accas saces
00564		t-c	- FCTFACALEU	allaalulla	quadagetta	
0000		· ~+~~~~~~~	састастска	Latauaaatt		59000000
00001	L L	. aaaaaaatacc	aaararooca	aadattudat	Cogacgacaa	CCCCCCCCCC
00044			ассспаспаа	aaaaacatcc	Cayactcauc	gattgtatt
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28981	l tgacttctgc	tcgctcacac	ctcactgraa	tataggeee	togatogttt	acaaaaccat
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2916	caaatgacaa	atctaccact	catggaaccg	gcacaactac	tttctctagc	agcagtgtcg
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		, adotadaca	atttccactt	caacaattat	Lattattyct	gcagcgacaa
				CCTACLACUL	: Clubbactac	ayaaaayaca
		- +~~+~~ <b>-</b> ++	~++>~++	' ACACCEAALI		
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		- + <i>~~~~</i> +	traramramt	adccacadca	accccagact	gtataggage
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2000		+	· accataatti	· catttttdai	t ataccccci	Lilyaliliy
2006	1	a taccesstacs	catoatoato	· cacaadacc	: adaddaacat	acticities
2010	1	o acatocaats	a ococtaatac	, attaccaaa	i tqaaccacaa	Colocatian
3018	1 tccctgcta	t tagttactto	aacctaaccg	g gcggagatga	a ctgaaacact	. caccaccicc

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~ ~ ~ ~ ~		accedence.	ппаасосото	occadauauc	LCayayacy C	Caicoaaaa
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31261	caaattgtgg	ttaatactaa	catatatata	gttggtgtat	cagacactgt	gaaccaaatg
31321	ggagggcttg	ttaatyytta	cotocootta	agattatatt	ttgactcttc	tggaaatcta
31381	ttcacacaaa	agacagcaaa	cacccaacca	cttaaaaata	aatcttctac	agcgaccagt
31441	ttaactgagg	aatcagactt	addadttcca	cccaaaaaca	caccttatcc	cttcaacacc
31501	gaaactgtag	ccagcagcaa	ageetttaty	ccaagtacta	agtacatgac	tagttatgat
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21/21		++~~~++~~	carrectata	atuctadaca	quuguatgat	CCCCCCCCC
24601		ccatacastt	taaataaat	ctaaacucaa	quaatttt	agaaagcaac
24541		+ ~ a ~ c a c a t c	CCCCEEEEC	EEEECLLaca	LLaLayaaya	cgacaacca
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24221		~~+~~~+	aarccarcgc	natacttt	Laaaucyccc	Loudageou
20041		atacaactcc	agaarrraga	CCACUULCAL	CLygaagaag	uucgucggg-
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32461	ctcttttggc	atgtgcatat	taacaatctg	tetgtaccat	ggacaacgee	ccatgcattg
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22021		- aacaacaaca	antantetto	: agtcatagaa	getegggtet	Catttette
20001		- aactoooctc	taatataaaa	r didaldiciu	gegeatgaty	ccgagegege
22041		· ctcataatdd	adttdcttcc	: toacaticic	glattigla	Lagcadaucg
22001		· araararart	CTTCTTCGCC	: ttctatcctq	Cogoliagog	Lychologic
22061		, atacadecae	- actcttaadi	LOUTCAAAAY	aatyctyyt	ccagccgcaa
22121	+ annongton	· atcocatcta	attottctda	a ggagatcatt	Cacygrayca	Lacycauace
22101		, satocaacto	: dattdcdtti	. caaocauuau	ayyayayyya	agagacggaa
33797	Ccaaccaage	. aatytttatt	ccaaacgat	tegeagtact	tcaaattgta	gatcgcgcag
33241	gaaccatgu	. torroccass	tatattaat	g aaaaagcaca	gctaaatcaa	aagaaatgcg
33301	. atggcatete	: Legecoccac	terettere	- casacctc	acgcgcacat	ccaagaacaa
33361	. attttcaagg	g tgctcaacgg		a caaaagccccc	atcatattac	attectocae
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33481	. cattcccaga	a taattttcag	CTTTCCagc	Ligantiali	. tanaanaa	cttgtggtaa
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22/01		a atmamaaat	· arcttdccc	c Editalcacci	, ytaytyaati	. gagaatggta
22551		acatoccctt	· aactctaaa	r rettetttaa	ı gılıcıagılı	Laaaaactt
22721		- caccaaacto	t cttagggag	a accccccq	gaacaayay	aggygacgcu
22701		- acaamemead	r accteccea	a ttooctcca	Caaaaacaac	j attyyaataa
22241		~ saccaccadi	- aatatcatc	a aagttactaa	I addididdi	: ayycayayıı
22221		, attmaataa:	adaaaaatt.	г оссалалада	i Calleaaaa	. cccagggatg
22061	l casatocas	t aggttacco	gctgcgctc	c aacattgtta	a gttttgaati	t agtctgcaaa
2370.	Liaaatytaa	- ~33	- 33-3			

34081 34141 34201 34261 34321 34381 34441 34501 34561 34681 34741	tttccatcac gattaaacaa catacaatcc gtataattat gcacaggaga gtccctctaa ggcacacaaa gccctaaact ccgaaactgc ttcctcttc	aaacaagcgt aagacaagcc cagcaccgaa agacatgtta gcttaatcgt ataaaaaata atacacatac ccacaagctc gacgtaatgg gtcaccaggg tcaccggtacg tttaaccgtt ttacatattg	acagggtete agttectege gcateagtta aagtatagca taattattte aaagceteat taaagteact gactaaagtg aaaagtacag tcacatecca	cagetegace ggtgaccage aggagaaaaa aagecacece tetgetgetg cagecatgge etceaacete taaaaaatec tttaacttce ttaacttaca ecaateaca	atgaataagt acagcaaca tcgcggatac tttaggcaac ttaccagaga tccacaatat cgccaaaccc gcaatccaa acgtcatttt cacggccac	cttgatgag tagcetttgg aaagtaaaag gtcgccccg aagtacagcg atatacacaa aacacacac caagcgtcac cccacggccg acttttaaa
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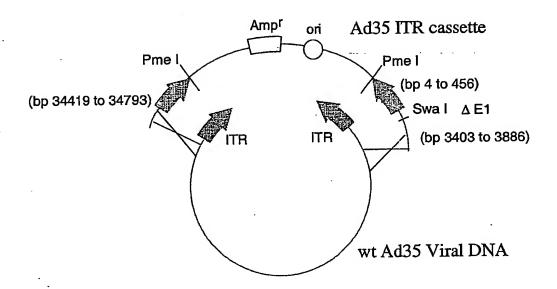


FIG. 3

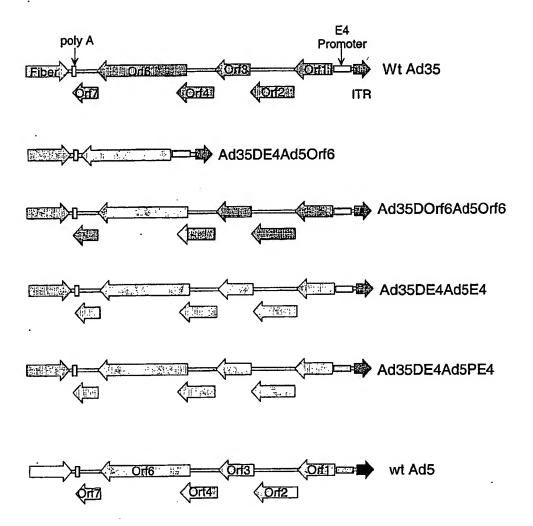
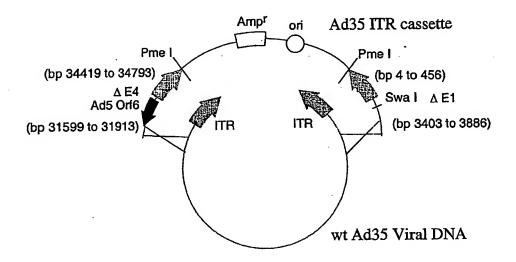


FIG. 4



# 15/59

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121	ttagttcata	gcccatatat	ggagttccgc	gttacataac	ttacggtaaa	tggcccgcct
181	ggctgaccgc	ccaacgaccc	cccccattg	acgtcaataa	tgacgtatgt	tcccatagta
241	acoccaatao	ggactttcca	ttgacgtcaa	tgggtggagt	atttacggta	aactgcccac
. 301	ttggcagtac	atcaagtgta	tcatatgcca	agtacgcccc	ctattgacgt	caatgacggt
361	aaatggcccg	cctggcatta	tgcccagtac	atgaccttat	gggactttcc	tacttggcag
421	tacatctacg	tattagtcat	cgctattacc	atggtgatgc	ggttttggca	gtacatcaat
481	gggcgtggat	agcogtttga	ctcacgggga	tttccaagtc	tccaccccat	tgacgtcaat
541	gggagtttgt	tttggcacca	aaatcaacgg	gactttccaa	aatgtcgtaa	caactccgcc
601	ccattgacgc	aaatgggcgg	taggcgtgta	cggtgggagg	tctatataag	cagagetegt
661	ttagtgaacc	gtcagatcgc	ctggagacgc	catccacgct	gttttgacct	ccatagaaga
721	caccaggacc	gatccagcct	ccacaaccaa	gaacggtgca	ttggaacgcg	gattccccgt
781	accaagagta	agatctaccA	TGGGTGCTAG	GGCTTCTGTG	CTGTCTGGTG	GTGAGCTGGA
841	CAAGTGGGAG	AAGATCAGGC	TGAGGCCTGG	TGGCAAGAAG	AAGTACAAGC	TAAAGCACAT
901	TGTGTGGGCC	TCCAGGGAGC	TGGAGAGGTT	TGCTGTGAAC	CCTGGCCTGC	TGGAGACCTC
961	TGAGGGGTGC	AGGCAGATCC	TGGGCCAGCT	CCAGCCCTCC	CTGCAAACAG	GCTCTGAGGA
1021	GCTGAGGTCC	CTGTACAACA	CAGTGGCTAC	CCTGTACTGT	GTGCACCAGA	AGATTGATGT
1081	GAAGGACACC	AAGGAGGCCC	TGGAGAAGAT	TGAGGAGGAG	CAGAACAAGT	CCAAGAAGAA
1141	GGCCCAGCAG	GCTGCTGCTG	GCACAGGCAA	CTCCAGCCAG	GTGTCCCAGA	ACTACCCCAT
1201	TGTGCAGAAC	CTCCAGGGCC	AGATGGTGCA	CCAGGCCATC	TCCCCCGGA	CCCTGAATGC
1261	CTGGGTGAAG	GTGGTGGAGG	AGAAGGCCTT	CTCCCCTGAG	GTGATCCCCA	TGTTCTCTGC
1321	CCTGTCTGAG	GGTGCCACCC	CCCAGGACCT	GAACACCATG	CTGAACACAG	TGGGGGCCA
1381	TCAGGCTGCC	ATGCAGATGC	TGAAGGAGAC	CATCAATGAG	GAGGCTGCTG	AGTGGGACAG
1441	GCTGCATCCT	GTGCACGCTG	GCCCCATTGC	CCCCGGCCAG	ATGAGGGAGC	CCAGGGGCTC
1501	TGACATTGCT	GGCACCACCT	CCACCCTCCA	GGAGCAGATT	GGCTGGATGA	CCAACAACCC
1561	CCCCATCCCT	GTGGGGGAAA	TCTACAAGAG	GTGGATCATC	CTGGGCCTGA	ACAAGATTGT
1621	GAGGATGTAC	TCCCCCACCT	CCATCCTGGA	CATCAGGCAG	GGCCCCAAGG	AGCCCTTCAG
1681	GGACTATGTG	GACAGGTTCT	ACAAGACCCT	GAGGGCTGAG	CAGGCCTCCC	AGGAGGTGAA
1741	GAACTGGATG	ACAGAGACCC	TGCTGGTGCA	GAATGCCAAC	CCTGACTGCA	AGACCATCCT
1801	GAAGGCCCTG	GGCCCTGCTG	CCACCCTGGA	GGAGATGATG	ACAGCCTGCC	AGGGGGTGGG
1861	GGGCCCTGGT	CACAAGGCCA	GGGTGCTGGC	TGAGGCCATG	TCCCAGGTGA	CCAACTCCGC
1921	CACCATCATG	ATGCAGAGGG	GCAACTTCAG	GAACCAGAGG	AAGACAGTGA	AGTGCTTCAA
1981	CTGTGGCAAG	GTGGGCCACA	TTGCCAAGAA	CTGTAGGGCC	CCCAGGAAGA	AGGGCTGCTG
2041	GAAGTGTGGC	AAGGAGGGCC	ACCAGATGAA	GGACTGCAAT	GAGAGGCAGG	CCAACTTCCT
2101	GGGCAAAATC	TGGCCCTCCC	ACAAGGGCAG	GCCTGGCAAC	TICCICCAGI	CCAGGCCTGA
2161	GCCCACAGCC	CCTCCCGAGG	AGTCCTTCAG	GTTTGGGGAG	GAGAAGACCA	CCCCCAGCCA
2221	GAAGCAGGAG	CCCATTGACA	AGGAGCTGTA	CCCCCTGGCC	atastatast	CCCTGTTTGG
2281	CAACGACCCC	TCCTCCCAGT	AAaacaaagc	cegggeagat	attenente	gracerece
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SEQ ID NO: 2

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121	ttagttcata	gcccatatat	ggagttccgc	gttacataac	tracygrada	tagatagta
181	ggctgaccgc	ccaacgaccc	ccgcccattg	acgtcaataa	tgacgtatgt	poetgeese
241	acgccaatag	ggactttcca	ttgacgtcaa	tgggtggagt	atttacggta	aactgcccac
301	ttggcagtac	atcaagtgta	tcatatgcca	agtacgcccc	ctattgacgt	taatgacggt
361	aaatggcccg	cctggcatta	tgcccagtac	atgaccttat	gggaetttee	caccigging
421	tacatctacg	tattagtcat	cgctattacc	atggtgatgc	ggttttggca	benestenat
481	gggcgtggat	agcggtttga	ctcacgggga	tttccaagtc	tecaceccat	coacyteaac
541	gggagtttgt	tttggcacca	aaatcaacgg	gactttccaa	aatgtegtaa	caacteegee
601	ccattgacgc	aaatgggcgg	taggcgtgta	cggtgggagg	tetatataag	cagagetegt
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721	caccgggacc	gatccagcct	ccgcggccgg	gaacggtgca	ttggaacgcg	gatteecegt
781	gccaagagtg	<u>a</u> gatcgatct	aagtaagctt	CCTGCATGCT	GCTGCTGCTG	CTGCTGCTGG
841	GCCTGAGGCT	ACAGCTCTCC	CTGGGCATCA	TCCCAGTTGA	GGAGGAGAAC	CCGGACTICI
901	GGAACCGCGA	GGCAGCCGAG	GCCCTGGGTG	CCGCCAAGAA	GCTGCAGCCT	GCACAGACAG
961	CCGCCAAGAA	CCTCATCATC	TTCCTGGGCG	ATGGGATGGG	GGTGTCTACG	GTGACAGCTG
1021	CCAGGATCCT	AAAAGGGCAG	AAGAAGGACA	AACTGGGGCC	TGAGATACCC	CTGGCCATGG
1081	ACCGCTTCCC	ATATGTGGCT	CTGTCCAAGA	CATACAATGT	AGACAAACAT	GIGCCAGACA
1141	GTGGAGCCAC	AGCCACGGCC	TACCTGTGCG	GGGTCAAGGG	CAACTICCAG	ACCATIGGCT
1201	TGAGTGCAGC	CGCCCGCTTT	AACCAGTGCA	ACACGACACG	CGGCAACGAG	GTCATCTCCG
1261	TGATGAATCG	GGCCAAGAAA	GCAGGGAAGT	CAGTGGGAGT	GGTAACCACC	ACACGAGTGC
1321	AGCACGCCTC	GCCAGCCGGC	ACCTACGCCC	ACACGGTGAA	CCGCAACTGG	TACTCGGACG
1321	CCCACCTCCC	TECCTCCECC	CGCCAGGAGG	GGTGCCAGGA	CATCGCTACG	CAGCTCATCT
1441	CCAACATGGA	CATTGACGTG	ATCCTAGGTG	GAGGCCGAAA	GTACATGTTT	CGCATGGGAA
1501	CCCCAGACCC	TGAGTACCCA	GATGACTACA	GCCAAGGTGG	GACCAGGCTG	GACGGGAAGA
1561	አጥርጥርርጥርር አ	GGAATGGCTG	GCGAAGCGCC	AGGGTGCCCG	GTATGTGTGG	AACCGCACTG
1621	AGCTCATGCA	GGCTTCCCTG	GACCCGTCTG	TGACCCATCT	CATGGGTCTC	TTTGAGCCTG
1601	CACACATCAA	ATACGAGATC	CACCGAGACT	CCACACTGGA	CCCCTCCCTG	ATGGAGATGA
17/1	CACACCCTCC	CCTGCGCCTG	CTGAGCAGGA	ACCCCCGCGG	CTTCTTCCTC	TTCGTGGAGG
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1021	CCCTCCTCAC	TGCCGACCAC	TCCCACGTCT	TCTCCTTCGG	AGGCTACCCC	CTGCGAGGGA
1 9 9 1	ርርጥ <u>ርር አ</u> ጥርጥቸ	· CGGGCTGGCC	CCTGGCAAGG	CCCGGGACAG	GAAGGCCTAC	ACGGTCCTCC
2041	<b>ጥአጥአ</b> ርርር እ እ እ	CCCTCCAGGC	TATGTGCTCA	AGGACGGCGC	CCGGCCGGAT	GTTACCGAGA
2101	CCCACACCCC	GAGCCCCGAG	TATCGGCAGC	AGTCAGCAGT	GCCCCTGGAC	GAAGAGACCC
2161	ACCCACCCGA	CCACCTGGCG	GTGTTCGCGC	: GCGGCCCGCA	GGCGCACCTG	GTTCACGGCG
2221	ጥርሮልሮርልሮርል	CACCTTCATA	GCGCACGTCA	TGGCCTTCGC	CGCCTGCCTG	GAGCCCTACA
2221	CCCCCTCCC	CCTGGCGCCC	CCCGCCGGCA	CCACCGACGC	CGCGCACCCG	GGTTAACCCG
23/1	tantecceae	attacttcct	ctactaacca	r ggacatcagg	tggcccccgc	tgaattggaa
2401	tcgatcagaa	ttgatctgat	ctactataca	: ttctagttgc	cagccatctg	ttgtttgccc
2461	ctcccccatc	r cetteettaa	ccctggaage	tgccactccc	actgtccttt	cctaataaaa
2521	rgaggaaatt	gcatcgcatt	gtctgagtag	, gtgtcattct	attctggggg	gtggggtggg
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FIG. 7

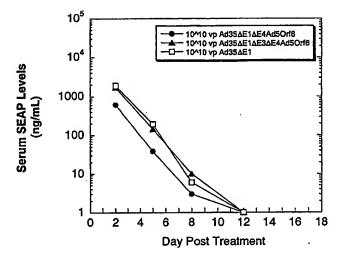
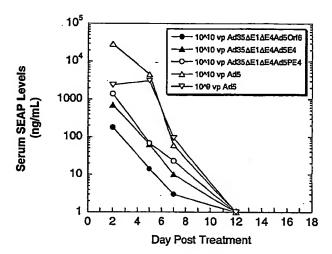


FIG. 8



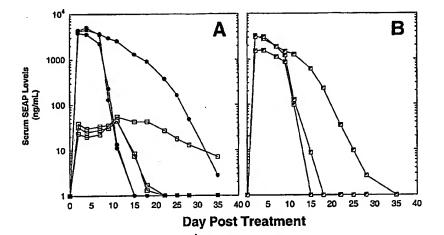


FIG. 10A-B

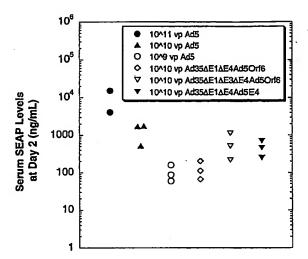


FIG. 11

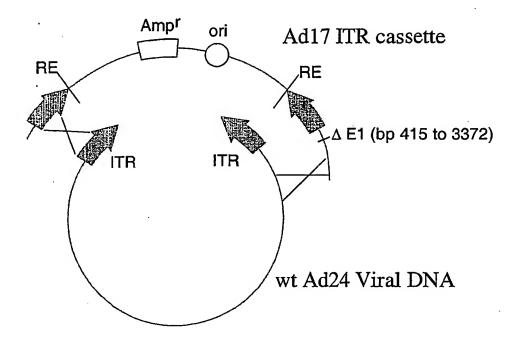


FIG. 12

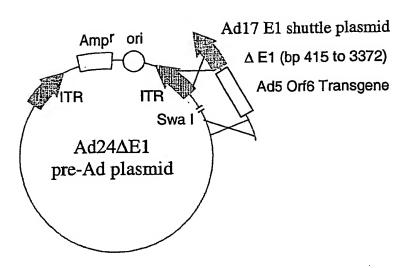


FIG. 13

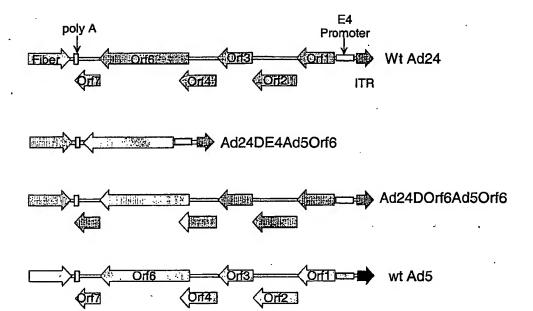


FIG. 14

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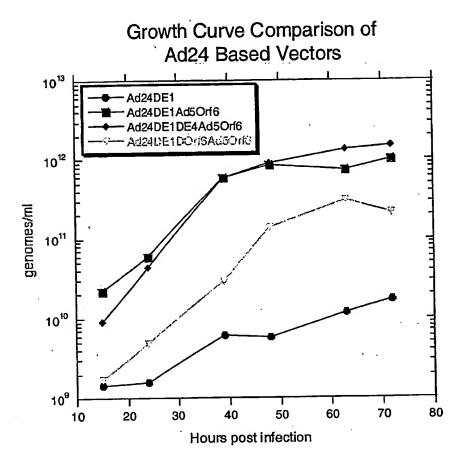


FIG. 15

	_					
1	catcatcaat	aatatacccc	acaaagtaaa	caaaagttaa	catgcaaatg	agettttgaa
61	tttagggcgg	ggccagcgct	gattggacga	gagaagatga	tgcaaatgac	gtcacgacgc
121	acggctaacg	gtcgccgcgg	aggcgtggcc	tagcccggaa	gcaagtcgcg	gggctgatga
181	cgtataaaaa	agcggacttt	agacccggaa	acggccgatt	ttcccgcggc	cacgcccgga
241	tatgaggtaa	ttctgggcgg	atocaaotaa	aattaggtca	ttttggcgcg	aaaactgaat
201	gaggaagtga	assatassa	ataccogtcc	cacccaaaac	ggaatattta	ccaaaaacca
301	agagactttg	adagegaaaa	tagagatttc	asttacaata	ttttttcaca	aatttccccc
201	tccgtgtcaa	accyattacy	thatatasas	gattgaggtg	tecacagog	atttaaacca
421	tccgtgtcaa	agteeggtgt	ttatyttata	gattagttga	acacagggt	standstoca
481	gtcgagcccg	tcaagaggcc	actettgagt	gecagegage	agagatttet	tractattes
541	ctcccagagt	ctgagaaaaa	tgagacacct	gegeeteett	tetteaaetg	Lycclattya
601	catggccgca	ttattgctgg	aggattatgt	gagtacaata	ttggaggacg	aactgcatec
661	atctccattt	gagctgggac	ctacacttca	ggacctatat	gatttggagg	tagatgccca
721	tgatgacgac	ccgaacgaag	aggctgtgaa	tttaatattt	ccagaatctc	tgattcttca
781	ggctgacata	gccagcgaag	ctgtacctac	accacttcat	acaccgactc	tgtcacccat
841	acctgaattg	gaagaggagg	acgagctaga	cctccgatgt	tatgaggaag	gttttcctcc
901	cagcgattca	gaggacgaac	agggtgagca	gagcatggct	ctaatctcaa	aatatgcttg
961	tataattata	gaagagcatt	ttgtgttgga	caatcctgag	gtgcccgggc	aaggctgtag
1021	atcctgccag	taccaccggg	ataagaccgg	agacacgaac	gcctcctgcg	ctctgtgtta
1081	catgaaaaag	aacttcagct	ttatttacag	taagtggagt	gaatgtgaga	gagactgagt
1141	gcttaacaca	taactgggta	atocttaaac	agctgtgcta	agtgtggttt	atttttgttt
1201	ctaggtccgg	tatcagagga	tgagtcatca	ccctcagaag	aagaccaccc	gtgtcccct
1201	gagetgteag	caccagagga	cctacaaata	cacagaccca	ccccagtcag	acccagtggc
1201	gagaggcgag	gegadaegee	aaaaattaaa	gacttottac	atgacatggg	tagagataaa
1321	cctttggacc	tagetytega	addacegag	aactagggt	acctotoctt	agtcatgtgt
1381	aaataaagtt	cyagereyaa.	acyccccagg	acceaggere	agtataattt	atractcatr
1441	aaataaagtt	gtacaataaa	agtacatgcg	acgeacgeaa	tacacacaca	acguecoucg
1501	ggcgtggctt	agtectatat	aagtggcaac	accugggcac	cygygcacag	accettatag
1561	agttcctgat	ggatgtgtgg	actatecttg	cagactttag	caagacacgc	cygettytag
1621	aggatagttc	agacgggtgc	teegggttet	ggagacactg	gcccggaacc	ectetatete
1681	gtctggtgta	cacagttaag	aaggattata	acgaggaatt	tgaaaatctt	tttgctgatt
1741	gctctggcct	gctagattct	ctaaatctcg	gccaccagtc	ccttttccag	gaaagggtac
1801	tccacagcct	tgatttttca	agcccagggc	gcactacagc	cggggttgct	tttgtggttt
1861	ttctggttga	caaatggagc	cagaacaccc	aactgagcag	gggctacatt	ctggacttcg
1921	cagccatgca	cctgtggagg	gcatgggtga	ggcagcgggg	acagagaatc	ttgaactact
1981	ggcttataca	gccagcagct	ccgggtcttc	ttcgtctaca	cagacaaaca	tccatgttgg
2041	aggaagaaat	gaggcaggcc	atggacgaga	acccgaggag	cggcctggac	cctccgtcgg
2101	aagaggagct	ggattgaatc	aggtatccag	cctgtaccca	gagcttagca	gggtgctgac
2161	atccatggcc	agggagtga	agagggagag	gagcgatggg	ggcaataccg	ggatgatgac
2221	cgagctgacg	accaacctaa	tgaatcgcaa	gcgtccagag	cgcattacct	ggcacgagct
2221	acagatggag	tatagggata	aggtgggcct	gatgcaggat	aaatatggcc	tagagcagat
23/11	aaaaacccac	taattaaacc	cagatgagga	ttgggaggag	gccattaaga	aatatoccaa
2741	gatagccctg	caccagatt	acaantacan	gatgaccaag	acogtgaata	tcagacatgc
2401	ctgctacatc	teagagaea	acacacacat	ggtcatcgat	accetggaca	aggccgcctt
2401	caggtgttgc	otenteres.	tasasaaaa	agtoatogat	atraattrca	tgattttcat
25ZI	caggigity	atyatyyyaa	cyagageegg	tagagataata	ttcatcacca	acactcacat
258I	gaacatgaag	ttcaatggag	agaayırıaa	cggggcgacg	tacacagaca	totagecacae
2641	gaccctgcac	ggctgcagtt	tetteggett	caacaacacg	rgcycayayy	cocygygege
	tgctaagatc					
2761	gagcgagatg	tctgtgaagc	agtgtgtgtt	tgagaaatgc	tacetgggag	tetetaeega
2821	gggcaatgct	agagtgagac	attgctcttc	cctggagacg	ggctgcttct	gcctggtgaa
2881	gggcacagcc	tctctgaagc	ataatatggt	gaagggctgc	acggatgagc	gcatgtacaa
2941	catgctgaca	tgcgactcgg	gggtctgcca	tatcctgaag	aacatccatg	tgacctccca
3001	ccccggaag	aagtggccag	tgtttgagaa	taacctactg	atcaagtgcc	acatgcacct
3061	gggcgccaga	aggggcacct	tccagccgta	ccagtgcaac	tttagccaga	ccaagctgct
3121	gctggagaac	gatgccttct	ccagggtgaa	cctgaacggc	atctttgaca	tggatgtctc
3181	ggtgtacaag	atcctgagat	acgatgagac	caagtccagg	gtgcgcgctt	gcgagtgcgg
3241	gggcagacac	accaggatgc	aaccagtggc	cctggatgtg	accgaggagc	tgaggcccga
3301	ccacctggtg	atggcttgta	ccgggaccga	attcaactcc	agtggggagg	acacagatta
3361	gaggtaggtt	gagtattagt	gaacataact	aaggtgacta	taaaggcggg	tgtcttacga
3/31	gggtctttt	actttata	agacatcato	aacqqqactq	acaggacett	casadaaaaa
3401	ctttttagcc	cttatttaa	aacccccctg	ccadastada	ccadaattca	tcagaatgtg
3E41	atgggatcga	contrasca	accepticity	cttccaccaa	attectedae	catgacctac
3341	acgygaccya	cygcygacyg	actentages	accacacaca	CCCCCCACC	cacsaccacc
200T	gcgaccgtgg	ggaactegte	geregacage	accyccycay	cogoggeouge	Jacascosco

FIG. 16A-1

3661	atgacagcga	cgagactggc	ttcgagctac	atgcccagca	gcagcagtag	cccctctgtg
3721	cccagttcca	tcatcgccga	ggagaaactg	ctggccctgc	tggccgagct	ggaagccctg
3781	agccgccagc	taaccaccct	gacccagcag	gtgtccgagc	tccgcgaaca	gcagcagcag
3841	caaaataaat	gattcaataa	acacagattc	tgattcaaac	agcaaagcat	Ctttattatt
3901	tattttttcg	cococootao	accetaatee	acctctcccg	atcattgaga	gtgcggtgga
3961	tttttccag	gacccggtag	aggtgggatt	ggatgttgag	gtacatgggc	atgagcccgt
4021	cccaaaaata	gacctggcag	cactgcatgg	cctcgtgctc	taggategtg	ttgtagatga
4001	tccagtcata	acadadacac	tagacataat	gctggatgat	gtccttgagg	aggagactga
4141	tagagagaga	geaggggege	atatacatat	tggcgaagcg	attaaactaa	gagggatgca
4741	taggedaeggg	gageeeeeg	actttccct	ggatcttgag	attaacaata	ttgccaccca
4201	cgcgggggga	gacgacgegg	ttatacaaa	ccaccagaac	gatatagece	gtgcacttgg
4201	gateeegeet	atacaactta	raarraatr	cgtgaaagaa	tttggagacg	cccttatacc
4321	ggaacttytt	ttacatacac	testeestes	tgatggcgat	adacccataa	actacaactt
4381	cacccaggui	atttataaa	taacacacat	cgtaattatg	ctcctagata	agatcatcat
4441	tggcaaagac	gutucugggg	ccagagacac	tgccagattg	gggggggg	attecetega
4501	aagacatttt	aatgaatttg	toacatattt	gcatctccca	gggguouatg	tcagaagaga
4561	geeeegggge	gaagtteece		aaacggtttc	ggcccccaca	gtgatgaggt
4621	ggatcatgtc	cacctgcggg	gcyacyaaaa	adacygcccc	cccatcaa	ccatagatas
4681	gcgaggagag	caggtttctc	aacagctggg	acttgccgca	cccggccggg	teceagaega
4741	ccccgatgac	gggttgcagg	tggtagttca	aggacatgca	ttagaggag	actcccggagga
4801	ggggggccac	ctcgttgagc	atgtetetga	cttggaggtt	recoggacy	ttcaccaccact
4861	ggaggcggtc	cccgcccagc	gagagcagct	cttgcaggga	agcaaagttt	negagagaga
4921	tgagcccgtc	ggccatgggc	atcttggcga	gggtctgcga	gaggagtteg	aggeggteet
4981	agagctcggt	gacgtgctct	acggcatctc	gatccagcag	acttectegt	cccgggggcc
5041	gggacgactg	cgactgtagg	gcacgagacg	atgggcgtcc	agegetgeea	gegeeatgee
5101	cttccagggt	ctcagtgtcc	gcgtgagcgt	ggtctccgtc	acggtgaagg	ggtgggeeee
5161	gggctgtgcg	cttgcaaggg	tgcgcttgag	actcatcctg	ctggtgctga	aacgggcacg
5221	gtcttcgccc	tgcgcgtcgg	cgagatagca	gttgaccatg	agctcgtagt	tgagggcctc
5281	ggcggcgtgg	cccttggcgc	ggagcttgcc	cttggaagag	cgcccgcagg	cgggacagag
5341	gagggattgc	agggcgtaga	gcttgggtgc	gagaaagacg	gactcggggg	cgaaagcatc
5401	cactccacaa	tagacacaga	cggtctcgca	ctcgaccagc	caggtgagct	cgggctgctc.
5461	ggggtcaaaa	accagttttc	ccccgttctt	tttgatgcgc	ttcttacctc	gcgtctccat
5521	gagtetgtgt	ccacactcaa	tgacaaacag	gctgtctgtg	tccccgtaga	cggacttgat
5581	gaacctatcc	tacagggggg	tecegeggte	ctcctcgtag	agaaactcgg	accactctga
5641	dacdaaddcd	cacatecaca	ccaagacaaa	ggaggccacg	tgcgaggggt	agcggtcgtt
5701	gtccaccagg	gggtccacct	tttccacggt	atgcagacac	atgtccccct	cctccgcatc
5761	caagaaggtg	attogcttot	aggtgtaggc	cacgtgaccc	ggggtccccg	acgggggggt
5821	ataaaaaaaa	acagatetat	actcatcctc	actctcttcc	gcgtcgctgt	ccacgagcgc
5991	carctattaa	ggtaggtatt	ccctttcgag	agcgggcatg	acctcggcac	tcaggttgtc
50/1	actttctaca	aacqaqqaqq	atttgatgtt	ggcttgccct	gccgcaatgc	tttttaggag
5001	agtttcatcc	atctootcag	aaaagactat	ttttttattg	tcaagcttgg	tggcgaagga
6061	accatagaga	acattagaga	gaagettgge	gatggatctc	atggtctgat	ttttgtcacg
6121	gecatagagg	teettaacea	castattasa	ctggacatac	tcgcgcgcga	cgcacttcca
6101	ttaggaraga	acaataataa	actentegra	cacgatectg	acgcgccagc	cgcggttatg
0101	caggggaag	acggeggege	taataaccac	ctcgccgcgc	aggggctcgt	togtccagca
6241	cagggrgace	agattcacgc	accadaacdd	gggcagcaca	tcaagcagat	actcatcaga
6301	gaggegreeg	teastaatas	ageuguuegg	acagagttco	ttotcaaaat	aatcgatttt
0207	ggggtetgta	testeessee	agatgtccgg	ctcgcgggcg	accaacactc	actcatagga
6421	tgaggatgta	carcaagg	ggatgggatg	cgtcagggcg	gagagagaca	taccacagat
6481	gttgaggggd	togaccccayy	gcacgggacg	accastated	rataaaataac	agcgccccc
6541	gtegtagaca	. Lagalgggel	ccyayayyac	geegaegeag	gegggaeaa	addcadaacc
6601	geggatgetg	gegegeaegt	agicalacaa	ctcgtgcgag	taacaaaaa	taccatacaa
6661	. gagattggtg	egetgggget	geteggegeg	gaagacgatt	. cggcgaaaga	tggcatgcga
6721	. gttggaggag	atggtgggcc	gttggaagat	gutaaagugg	gcargaggca	gacgaaccga
6781	. gtcgcggatg	aagtgegegt	aggagtettg		acyayeeegy	cggtgacgag
6841	. gacgtccatg	gcgcagtagt	ccagcgtttc	geggatgate	toataacccg	cctctccttt
6901	. cttctcccat	agctcgcggt	tgagggcgta	ctcctcgtca	teetteeagt	actcccggag
6961	cgggaatcct	cgatcgtccg	cacggtaaga	geceageate	, tagaaatggt	tcacggcctt
7021	gtagggacag	cagcccttct ct	ccacggggag	, ggcgtaagct	: tgagcggcct	tgcggagcga
7081	aatatacata	agggggaagg	tatccctgac	: catgactttc	: aagaactggt	acttgaaatc
7141	cgagtcgtcg	r caqccqccqt	gctcccagag	, ctcgaaatc	g gtgcgcttct	: tcgagagggg
7201	gttaggcaga	ι σεσαααστσα	cqtcattgaa	n gagaatette	g cetgeeegeg	f gcatgaaatt
7261	L gcgggtgatg	g cggaaagggc	ccgggacgga	a ggctcggttg	g ttgatgacct	gggcggcgag

FIG. 16A-2

7221	~~a~~t~t~~	tcgaagccgt	tratattata	cccgacgatg	tagagttcca	tgaatcgcgg
1321	gacgaceteg	atgtgcggca	acttttaa	ctcctcataa	gtgaggtcct	cgggggaatg
7381	geggeettta	tgctcgagcg	getettegag	asastataaa	ttaacttaca	tgaatgaage
7441	cagtccgtgc	tgctcgagcg		atactacas	ecaaccaca	actoctoocc
7501	ccagageteg	cgggccataa	gggtetggag	~ and at a sec	aagaggegga	cccaccatc
7561	cacggccatc	tttctgggg	tgacgcagta	gaaagcaagg	aggiccigci	cccagagaca
7621	ccagcgtaag	cgcacggcta	gategegage	gagggegaee	agetetgggt	agetatagat
7681	tttcataacc	agcataaagg	ggacgagctg	cttgccgaag	gaccccatcc	aggiglaggi
7741	ttctacatcg	taggtgacaa	agagccgctc	cgtgcgagga	tgagagccga	ttgggaagaa
7801	ctggatttcc	tgccaccagt	tggacgagtg	gctgttgatg	tgatgaaagt	agaaateeey
7861	ccaacaaacc	gaggagtcgt	actaatactt	gtaaaagcgt	ccgcagtact	cgcagcgctg
7921	cacqqqctqt	acctcatcca	caagatacac	agcgcgtccc.	ttgaggagga	acttcaggag
7981	tageageest	aactaataat	tttcatgttc	gcctgcgtgg	gactcaccct	ggggeteete
8041	daddacddad	aggetgaega	acccacacaa	gagccaggtc	cagatctcgg	cgcggcgggg
8101	acadagaaca	aagacgaggg	cacacaatta	ggagctgtcc	atggtgtcgc	ggagatecag
2161	atccagagae	agggttctga	agttgacctc	gtagaggcgg	gtgagggcgt	gcttgagatg
8221	cagatogtac	ttgatctcca	caggtgagtt	ggtggctgtg	tccacgcatt	gcatgagccc
8281	ataactacac	ggggccacga	ccataccaca	gtgcgctttt	agaagcggtg	tcgcggacgc
8341	acteceases	gcagcggcgg	ttccaaccc	gegggeaggg	gcggcagagg	cacgtcggcg
8/01	taccactcaa	gcaggtcccg	atactacacc	ctgagagcgc	tggcgtgcgc	gacgacgcgg
9461	caattaacat	cctggatctg	ccacctctac	gtgaagacca	ccggccccgt	gactttgaac
0501	ctcsacce	gttcaacaga	atcaatctcg	gcgtcattga	caacaaccta	acgcaggatc
0521	tetteesest	cgcccgagtt	atcatagtag	gcgatctcgg	acatgaactg	ctcgatctcc
0001	tertgeacge	gatcgccgcg	accededed	treacagtag	caacaaaatc	attogagatg
8541	teeteetgga	gctgcgagaa	gcccgcgcgc	ccactatcat	tccagacgcg	gctgtagacc
8 / O T	cgacccatga	cggcgtcgcg	ggcgcccagg	accacctcc	caaaattaaa	ctccacqtqc
8761	acgtccccgt	cggcgtagtt	cgcgcgcacg	tacaccagag	agtttagggt	ggtggcgatg
8821	cgcgtgaaga	eggegtagtt	gegeaggege	cadcacacaca	agetetaggge	gatgatgatg
8881	tgctcggtga	cgaagaagta	catgatecag	cygcycaggy	casacttass	aaactggggg
8941	atggcctcca	gcctttccat	ggeetegtag	adacccacag	taaattaaa	datactagges
9001	ttgcgggccg	agaccgtgag	etegteetee	testestet	catattatta	catracracc
9061	cgcacctcgc	gctcgaaatc	cccgggggcc	tt	CCCCCCCCCC	accaccacca
9121	tcttcttcta	tttcttcctc	tgggggcggt	ggcggcggcg	gggcccgacg	acgacygcgu
9181	cgcaccggga	gacggtcgac	gaagegeteg	accateteee	egeggeggeg	acycatyycz
9241	tcggtgacgg	cgcgaccccg	ttcgcgagga	cgcagcgtga	agaegeegee	tattatasat
9301	cggtaatggg	gcgggtcccc	gttgggcagc	gagagggcgc	tgacgatgca	thereares
9361	tgcggtgtag	gggacgtgag	cgcgtcgaga	tcgaccggat	eggagaatet	-t-cyayyaaa
9421	gcgtctagcc	aatcgcagtc	gcaaggtaag	ctcaaacacg	tageagecet	gradacte
9481	ttagaattgc	ggttgctgat	gatgtaattg	aagtaggcgt	ttttaaggcg	geggatggtg
9541	gcgaggagga	ccaggtcctt	gggtcccgct	tgctggatgc	gaagccgctc	ggccatgccc
9601	caggcctggc	cctgacaccg	gctcaggttc	ttgtagtagt	catgcatgag	cctctcaatg
9661	tcatcactgg	cagaggggga	gtcttccatg	cgggtgaccc	cgacgcccct	gagcggctgc
9721	acqaqcqcca	ggtcggcgac	gacgcgctcg	gcgaggatgg	cctgttgcac	gcgggtgagg
9781	gtgtcctgga	agtcgtccat	gtcgacgaag	cggtggtagg	ccccggtgtt	gatggtgtag
9841	atacaattaa	ccatgagcga	ccagttgacg	gtctgcaggc	cgggttgcac	gacctctgag
9901	tacctgagee	acaaaaaaac	gcgcgagtcg	aagacatagt	cgttgcaggt	gcgcacgagg
9961	tactootate	caactaggaa	atacaacaac	ggctggcggt	agagcggcca	gegergggrg
10021	accaacacac	ccaaaaccaa	atcetegage	atgaggcggt	ggtagccgta	gaggtagcgg
10081	gacatccagg	tgatgccggc	gacggtggtg	gaggcgcgcg	ggaactcgcg	gaegeggtte
10141	cagatottoc	gcagcggcag	gaaatagtcc	atggtcggca	eggtetggee	ggtgagacgc
10201	gracagteat	tgacgctcta	gaggcaaaaa	cgaaagcggt	tgagcgggct	cttcctccgt
10261	aggetageag	aacgcaaacg	gattagacca	catatatacc	ccggttcgag	tcccctcgaa
10201	tcacctcca	gccgcgacta	acgtggtatt	ggcactcccg	tetegaceeg	agcccgatag
10321	ccaggorgga	acggcggaga	accetttta	ccgaccgagg	ggagtcgcta	gacttgaaag
10701	ccgccayyat	ccccaccaaa	tagtggctcg	cacccataat	ctggagaagc	tttgccaggg
TO##T	thanktaga	gcagaacccg	attemenae	aaccacaaca	agcgggactt	gatcacccca
TOOUT	LEGAGLEGEG	geagaaceeg	cacccactt	ctccacttec	addadcdadc	cccttttt
10261	ccgatttaaa	gacccacage	atactacaca	aaatocotco	- cacccccct	ccaacaacca
10621	ctttttgcca	gatgcatccc	gecergegee	ctataaccc	accacaacaa	acagagatog
T0981	ccgcgaccgc	ggccgtagca	ggegeeggeg	+aaaaaaaaaa	gccacageag	COSCSCCCC
10741	acttggaaga	gggcgaaggg	ctggcgagac	cgggggcgcc	- tacaceagag	ctattanaa
10801	gcgtgcagct	gcagaaggac	gryegeeegg	egracytycc	ttttcccccc	ducadadada
10861	accgcagcgg	ggaggagccc	gaggagatgc	######################################	- coultyggig	22cagggage
10921	tgcgcgaggg	cctggaccgc	cagegegtge	Lgcgcgacga	gyarrrryay	ccyaacyage

FIG. 16A-3

10981	agacggggat	cagccccgcg	cgcgcgcacg	tggcggcggc	caacctggtg	acggcctacg
110/1	agragaragt	maaggaggag -	cacaacttcc	aaaagagttt	Caacaaccat	gracacac
11101	taatememen	caaggaggtg -	accctaaact	tgatgcacct	grgggactry	gcggaggcca
11101	taatcgcgcg	cccggacagc	aageetetga	caacacaact	attectagta	gtgcagcaca
TTT0T	ccgtgtagaa	cgaggcgttc	auduauucuc	toctaaacat	caccaaaccc	gagggccgct
11221	geagggaeaa	gctgatcaac	atottocada	getcatagt	gcaggagcgc	agcctgagcc
11281	ggctgctgga	getgateaac	attitudaga	controtage	cctadacaaa	ttttacgcgc
11341	tggccgagaa	ggtggcggct	accaaccacc	toggegeegag	aataaaaita	gacagetttt
11401	gcaagattta	caagacgccg	taegtgeeca	Lagacaagga	actacacata	taccacaaca
11461	acatgcgcat	ggcgctcaag	gtgctgacgc	tgagegaega	ectgggcgcg	caccacatas
11521	accgcatcca	caaggccgtg	agcgcgagcc	ggeggegega	getgagegae	tactacttca
11581	tgctgagtct	gcgccgggcg	ctggtagggg	gegeegeegg	eggrgaggag	gastaggets
11641	acatgggggc	ggacctgcat	tggcagccga	accaacacac	errggaggee	geetaeggee
11701	cadaddactt	ggatgaggat	gaggaggg	aggaggatgc	accegetgeg	gggtactgac
11761	gcctccgtga	tgtgtttta	gatgcagcaa	gccccggacc	ccgccataag	ggeggegetg
11221	caaagccagc	catccaatct	agcatcggac	gactgggagg	cegegatgea	acycattaty
11221	accetaacaa	cccacaaccc	cgagtccttt	agacaacagc	cgcaggccaa	Cayacteteg
11941	accattctag	aggcagtagt	ccctctcgg	accaacccca	cgcacgagaa	ggrgerggeg
12001	atcotoaaco	cactaacaa	gaacaaggcc	atccgtcccg	acgaggccgg	getggtgtae
12061	aacgccctgc	tagagggggt	gggccgctac	aacagcacaa	acgrgcagrc	caacciggac
12121	caactaataa	canacataca	caaaaccata	gcgcagcgcg	agcggttcaa	gaacgagggc
12181	ctaggetegt	taataacact	gaacgccttc	ctggcgacgc	agccggcgaa	cgtgctgtgt
12241	addcaddacd	attacaccaa	ctttatcagc	gcgctgcggc	tgatggtgac	cgaggigeee
12201	cadadcdadd	totaccagte	agacccagac	tactttttcc	agacgagccg	geagggeerg
12361	cadacddtga	acctaagcca	ggctttcaag	aatctgcgcg	ggctgtgggg	egigeaggeg
12/21	cccataggga	accootcoac	ggtgagcagc	ttgctaacgc	ccaactcgcg	gergergerg
12/81	ctoctoatco	caccetteac	cgacagcggc	agcgtgaacc	gcaactegta	CCLgggccac
125/1	ctactacac	tttaccccca	gaccatagge	caggcgcagg	tggacgagca	gacciccag
12601	gagatcacta	acataaacca	cacactaggt	cagaacgaca	ccgacagtct	gagagecace
12661	ctgaacttct	toctoacaaa	tagacagcag	aagattccgg	cgcagtacyc	gergreggee
12771	Geaggeree	gcatcctgag	atatotocao	cagagegtag	ggcttttcct	gatgcaggag
12721	addaccaccc	ccagcgccgc	actagacata	accgcgcgca	acatggaacc	tagcatgtac
12011	ggggccaecc	ggccgttcat	caataagctg	atggactacc	tgcaccgcgc	ggctgccatg
12011	aactcaact	actttactaa	toctatacta	aacccgcact	ggctcccgcc	gccggggttc
12061	tacacagaca	agtacgacat	gcccgacccc	aacgatgggt	tcctgtggga	cgacgtggac
12021	acacgggcg	tctccccgac	cttgcaaaag	caccadaada	cggtacgcac	gcccgcgagc
12021	agegeggege	tgggtcggag	cccctttcct	agcttaggga	gtttgcatag	cttgccgggc
T200T	tagggcgcgg	gcggcagggt	dadccddccd	cacttactaa	gcgaggacga	gtacctgaac
13301	gaataataa	tgcagccgcc	acaaatcaaa	aacgccatgg	ccaataacgg	gatagagagt
13201	gactegetge	aactgaaccg	ctacaaaacc	tacgctcagg	accataggga	tgcgcccgcg
13201	ctggtggaca	agcgccacga	ccggaagaca	gacctgatat	gggacgacga	ggactcggcc
13321	cegeggegae	gcgtgttgga	cttaaacaa	ancoatagg	ccaacccqtt	cgcgcatctg
13381	gacgatagea	tggggcgacg	cetgggeggg	atcaaataaa	actcaccaag	gccatagcgt
13441	cageceagae	ccttgttaga	gatgettega	acgataatat	cttcctctcc	tecteceteg
13501	gegttetett	testaga	gatgaggege	geggeggege	tratacetee	gcggtatatg
13561	tacgagagcg	tgatggcgca	ggcaaccccg	tactcccac	taactccaca	gtacgacacc
13621	. gctcctacgg	agggcagaaa	caycattegt	- caccegguae	cttccctgaa	ctaccaaaac
13681	. actcgcgtgt	acttggtgga	caacaagucg	geggaeaceg	atttcaccc	caccagaacc
13741	gaccacagea	acttcctgac	cacggraggra	tagaacaacg	, accordant	rascaccatt
13801	. agcacgcaga	cgataaattt	tgacgagcgg	tegeggtggg	geggegaett	taagaccacc
13861	L ctgcacacca	acatgcccaa	tgtgaacgag	tacatguica	ccaycaayc	caaggegegg
13921	L gtgatggtgg	ctaggaaggt	ggtagatcag	aatgatayy	. gcaaggacga	gitadaatat
13981	L gagtggtttg	agtttaccct	gcccgagggc	aacttttccg	agaccatgac	catagacciy
14041	atgaacaacg	ccatcttgga	aaactacttg	caagtgggg	ggcaaaatgg	cytyctygay
14101	L agcgatatco	gagtcaagtt	tgacagcagg	aatttcaago	. cgggctggga	tetestasts
14161	L aagctggtga	tgcctggggt	ctacacctac	gaggccttc	accoggacgt	. egegetgetg
1/221	ceanactace	, gagtagaett	caccgagagg	cacctgagca	a acctectggg	, cattegeaay
1 / 2 0 1	l sagraaccti	- tccaagaggg	cttcaggato	: atotatgagg	g atctcgaggg	gegraacate
1 / 2 / 1	Lecenceste	- togatotcaa	gcaatattt	r gatagtaaa	a agaagcttga	a ggaggcaaca
1 4 4 0 1	l cacastors:	- ccagggctgc	togagatato	agaggagac	a gicalatico	: aagagctgtg
1 446	l daadaadcd	r ctgaaaagga	tetaateatt	gtaccagta	a cacaagatga	a aagtaagaga
1 / 501	1 aggtataat	, tcatagatga	r cacccatgac	: accetetace	c gaagttggta	a cctgtcctat
14583	l acctacqqq	g accccgagaa	gggggtgcag	tcgtggacg	c tgctcaccad	c cccggacgtc

FIG. 16A-4

14641	acctgcggcg	cggagcaagt	ctactggtcg	ctgccggacc	tcatgcaaga	ccccgtcacc
14701	ttcccctcta	cccagcaagt	cagcaactac	cccgtggttg	gcgccgagct	catgcccttc
14761	cacaccaaga	gcttttacaa	cgacctcgcc	gtctactccc	agctcatccg	cagctacacc
14821	teceteacce	acotetteaa	ccgcttcccc	gacaaccaga	tectetgeeg	teegeeegeg
14881	cccaccatca	ccacggtcag	tgaaaacgtg	cctgctctca	cagatcacgg	gacgctaccg
14941	ctgcgcagca	gtatccgcgg	agtccagcga	gtgaccgtca	ctgacgcccg	tcgccgcacc
15001	totecetaco	tctacaaggc	cctgggcata	gtcgcgccgc	gcgtgctttc	cagtcgcacc
15061	ttctaaaaaa	tototattct	catctcccc	agcaataaca	ccggctgggg	tcttactagg
15121	CCCaucacca	tatacagaga	agccaagaag	cactcccaac	agcaccccgt	ccacatccac
15121	accepttcc	acactcccta	agacacttac	aagcgcgggc	ggacttctac	caccaccata
15241	cacaccacca	tcaccacct	catcgactcg	ataatcacca	acgcgcgcaa	ctataccccc
15301	ccccctcca	ccatagacge	agtcatcgac	agcatagtag	ccgacgcgcg	caactatacc
15361	acacccccca	accaacaaca	acquatcqcc	aggcgccacc	ggagtacgcc	caccatacac
15/21	agacgcaaga	ctctactaca	ccacaccada	cacacaaacc	gccgggccat	gatgcgagcc
15421	geegeeeggg	ccaccactac	accccccca	ggcaggactc	gcagacgagc	aaccaccacc
155/1	gegegeegeg	ccatttctag	catraccara	cccaaacaca	gaaacgtgta	ctaaatacac
15601	googeogegg	caacataca	cataccata	cacacccatc	ctcctcgtcc	ctgatctaat
12001	gattetetea	taggegegeg	acascastat	caaaacacaa	aatcaaggag	gagatgetee
15721	gertgegeee	ccccccgcaa	tacccac	cccaaacaaa	ccagaaaccc	cocaaaatca
15701	aggregrege	cccygagact	racgyaccac	acconggegga	agagtttgtg	cacaaattca
15/01	aycyyyttaa	aaaaaaayyat	tacasaaaa	agggggage	gcgcgtgttg	caacccaaca
15001	eccegeggeg	gtytytaaat	racasacant	cctcaatcaa	gagcaagcgt	agctatgacg
T220T	eggeggegge	gritacyccc	ggcgagcggc	agggggggg	gegggeggge	gattcacct
12301	aggtgtacgg	cyacyacyac	accetygace	totoottoo	gctggacgag	acceaccca
16021	acgggaagcg	geegegegaa	gaggagetga	agatastass	ccaaccacta	ctactacca
10081	cgcctagcct	gaageeegtg	accetgeage	tataggggg	ccaagcagtg	ataatacca
16141	geegeggggt	caagegegag	gycyagaaca	cactanana	catgcagatc	acggegeeea
16201	agegeeggeg	cgtggaagaa	gtgctggaca	ccytyaaaat	ggatgtggag	cccgaggeca
16261	aggtgcgccc	.catcaagcag	grggegeegg	geergygege	gcagaccgtg	gacacccaga
16321	tececacega	catggatgtt	gacaaaaaac	cetegaceag	catcgaggtg	cagaccgacc
T038T	cetggeteec	agectecace	getgeegtet	ccacciccac	cgccgccacg	gttattgatg
16441	ctcccagaag	gcgaagatgg	ggeeetgeea	accygetyat	gcccaactac	gractycatc
16501	cttccattat	cccgacgccg	ggctategeg	geaceeggta	ctacgccagc	cycayycycc
16561	cagccagcaa	acgccgccgc	cgcaccgcca	ceegeegeeg	tetggeecee	toggegege
16621	gccgcgtaac	cacgcgccgg	ggeegetege	tegttetgee	caccgtgcgc	caccacccca
16681	gcatccttta	atccgtgtgc	tgtgatactg	ttgcagagag	atggctctca	tereserves
16741	gcgcatcccc	gtcccgaatt	accgaggaag	atecegeege	aggagaggca	cggcaggcag
16801	cggcctcaac	cgccgccggc	ggegggeeat	gegeaggege	ctgagtggcg	getttetgee
16861	cgcgctcatc	cccataatcg	cggcggccat	cggcacgatc	ccgggcatag	etteegttge
16921	gctgcaggcg	tcgcagcgcc	gttgatgtgc	gaataaagcc	tctttagact	etgaeacaec
16981	tggtcctgta	tatttttaga	atggaagaca	teaattttge	gtccctggct	eegeggeaeg
17041	gcacgcggcc	gttcatgggc	acctggaacg	agatcggcac	cagccagctg	aacgggggcg
17101	ccttcaattg	gagcagtgtc	tggagcgggc	ttaaaaattt	cggctcgacg	ctccggacet
17161	atgggaacaa	ggcctggaat	agtagcacgg	ggcagttgtt	aagggaaaag	ctcaaagacc
17221	agaacttcca	gcagaaggtg	gtggacggcc	tagcctcggg	cattaacggg	grggrggaca
17281	tagcaaacca	ggccgtgcag	cgcgagataa	acagccgcct	ggacccgcgg	ccgcccacgg
17341	tggtggagat	ggaagatgca	actecteege	cgcccaaggg	cgagaagcgg	ccgcggcccg
17401	acgcggagga	gacgatcctg	caggtggacg	agccgccctc	gtacgaggag	gccgtcaagg
17461	ccggcatgcc	caccacgcgt	atcatcgcgc	cactggccac	tggtgtaatg	aaacccgcca
17521	cccttgacct	gcctccgcca	cccacgcccg	ctccaccgaa	ggcagctccg	gttgtgcagc
17581	cccctcctgt	ggcgaccgcc	gtgcgccgcg	tccccgcccg	ccgccaggcc	cagaactggc
17641	agagcacgct	gcacagtatc	gtgggcctgg	gagtgaaaag	tctgaagcgc	cgccgatgct
17701	attgagagag	aggaaagagg	acactaaagg	gagagcttaa	cttgtatgtg	ccttaccgcc
17761	agagaacgcg	cgaagatggc	taccccctcg	atgatgccgc	agtgggcgta	catgcacatc
17821	gccgggcagg	acgcctcgga	gtacctgagc	ccgggtctgg	tgcagtttgc	ccgcgccacc
17881	gacacgtact	tcagcctggg	caacaagttt	aggaacccca	cggtggctcc	cacccacgat
17941	gtgaccacgg	accggtccca	gcgtctgacg	ctgcgctttg	tgcccgtgga	tcgcgaggac
18001	accacgtact	cgtacaaggc	gcgcttcact	ctggccgtgg	gcgacaaccg	ggtgctagac
18061	atggccagca	cttactttga	catccgcggc	gtcctggacc	gcggtcccag	cttcaaaccc
18121	tactcgggca	cggcttacaa	cagcctggcc	cccaaaggcg	cccccaactc	tagtcagtgg
18181	gaacaagcta	aagctaccaa	tgccggtcaa	aaggaaactc	acacatttgg	agtagccgct
18241	atgggcggag	aagacattac	agtgaaaggt	cttcaaattg	gaactgatga	aactaaggaa
					•	

FIG. 16A-5

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18301	gatggagagg	atgaaatttt	tgcagatcaa	acattccagc	cagaacctca	agrgggagaa
19361	caraactroc	aagaaacgtt	tottttctat	ggaggcagag	ctcttaagaa	agaaaccaaa
10301	cagaaccggc		ttataaaaa	cccacaaatg		acaggetaaa
18421	atgaagccat	gttatggete	ccacycyaya	Cccacaacy	aaaagggagg	~~~+++
18481	tttacacttg	atgaaaaagg	tcagccaacc	aaaattcctg	atattacaat	ggatttttt
18541	gatagtccac	aagatgatac	atcaggtgta	actaataagc	cagatattgt	catgtatgca
10601	~====tat==	atttamaamo	tectoacaca	catgtagttt	acaaaccagg	caaagatgat
10001	yaaaatytaa	acctagaage		acceptagete	acacacccaa	ctacatcggg
18661	tctagttctt	ccgctaacct	cacacaacag	gccatgccta	acayaccyaa	
18721	ttcagagaca	actttgtggg	tcttatgtac	tacaatagta	ctggcaacat	gggtgtgttg
18781	actaatcaaa	cctctcagtt	gaatgctgtg	gtcgacttgc	aagacagaaa	caccgagctg
100/1	tottaccacc	tattoctaga	ttctctagat	gacagaacca	gatactttag	catgtggaat
10041	t-t-accage	tata	accessing	accatcatta	adaatcacdd	tatagaagat
TRAOT	tctgcagtgg	acagetatya	cecegacyce	aggatcattg	-to	2525344
18961	gaacttccaa	actattgctt	cccactgaat	ggcagtggtt	ctaacagcac	acacaaggc
19021	gttaaagctg	gaactggaaa	caattgggat	gacgatgaaa	atgttgcaag	acaaaatcag
19081	attoucacto	gcaacctgtt	caccatagaa	atcaacctcc	aggccaacct	atggaagagt
10141	this this is	goddoorgoo	agtataaata	cccgactcct	acaantacac	accaaccaac
19141	tttetgtact	cgaacguggc	CCLycaccig			gatagoonec
19201	gtcacgctgc	ccaccaacac	caacacctac	gactacatga	acggeegege	ggtagecee
19261	tegetggtgg	acgcctacat	caacattggc	gcccgctggt	cgctggaccc	catggacaat
19321	gtcaatccct	tcaaccacca	ccacaacaca	ggcctgcgct	accgctccat	gctcctgggc
10201	aacaacaact	acataccett	ccacatccaa	gtgccccaaa	agttctttgc	catcaagaac
13301	aacygccyct	acgugactu	-tt-a	gogocodaa	toccoage	catcaacata
19441	ctgcttctgc	receeggite	CLacacctac	gagtggaact	tttgtaagga	cgccaacacg
19501	atcctgcaga	gttccctcgg	caacgacctg	cgcgtcgacg	gegeeteegt	cegettegae
19561	agcotcaacc	tctacgccac	cttcttcccc	atggcgcaca	acaccgcctc	caccctggaa
19621	accatactac	graacgacac	caacgaccag	tccttcaacg	actacctctc	ggccgccaac
10.001	stastatasa	goadogacac	caacaccacc	aacgtgccca	tetecatece	ctcgcgcaac
TA09T	acgeterace	Cyatectegge	caaggccacc	ancytyccca	222222222	teceteete
19741	tgggccgcct	teegeggetg	gagtttcacc	cggctcaaga	ccaayyaaac	
19801	ggctcgggtt	tcgaccccta	ctttgtctac	tcgggctcca	tcccctacct	cgacgggacc
19861	ttctacctca	accacacctt	caagaaggtc	tccatcatgt	tcgactcctc	ggtcagctgg
10021	cccaacaaca	acconctact	cacgccgaac	gagttcgaga	tcaagcgcag	cattaacaga
19921	cccggcaacg	accegeceec	ategooguse	accascoscs	anttectent	ccagatgctc
19981	gagggctaca	acgrggccca	augeaacaug	accaaggact	ggtttttt	
20041	tcccactaca	acatcggcta	ccagggcttc	cacgtgcccg	agggctacaa	gyaccycacy
20101	tactccttct	tccgcaactt	ccagcccatg	agcaggcagg	tggtcgatga	gatcaactac
20161	aaggactaca	aggccgtcac	cctacccttc	cagcacaaca	actcgggctt	caccggctac
20101	attacasas	aggeogean	addaced	taccccgcca	acttccccta	cccactcatc
20221	ectigogodda	Clargeycea	ggggcagccc	caccegeea	teteesses	agtestates
20281	ggctccaccg	cagttccctc	cgrcacccag	aaaaagttcc	Letycyacay	ggccacgcgg
20341	cgcatcccat	tctccagcaa	ctttatgtcc	atgggcgccc	tcaccgacct	gggtcagaac
20401	atoctctato	ccaactcggc	ccacgcgctc	gacatgacct	ttgaggtgga	ccccatggat
20461	asaccesece	tectetatet	tetettegaa	gttttcgacg	tootcagagt	gcaccagccg
20401	gageceaece	tastagaga	catatacata	cgcacgccct	tetecaceaa	caacactacc
20521	caccgcggcg		Cytctactty	cycacycccc		
20581		ccasegagge				gagatagat
	acttaagcat	gagcggctcc	agcgaacaag	agctcgcggc	catcgtgcgc	gacctgggat
20641	acttaagcat gcgggcccta	gagcggctcc ctttttggga	agcgaacaag acccacgaca	agctcgcggc agcgcttccc	catcgtgcgc tggcttcctt	gacctgggat gccggcgaca
20641	acttaagcat gcgggcccta	gagcggctcc ctttttggga	agcgaacaag acccacgaca	agctcgcggc agcgcttccc	catcgtgcgc tggcttcctt	gacctgggat gccggcgaca
20701	acttaagcat gcgggcccta agctggcctg	gagcggctcc ctttttggga cgccatcgtc	agcgaacaag acccacgaca aacacggccg	agctcgcggc agcgcttccc gccgcgagac	catcgtgcgc tggcttcctt cggaggcgtg	gacctgggat gccggcgaca cactggctcg
20701 20761	acttaagcat gcgggcccta agctggcctg cctttggctg	gagcggctcc ctttttggga cgccatcgtc gaatccgcgc	agcgaacaag acccacgaca aacacggccg tcgcgcacct	agctcgcggc agcgcttccc gccgcgagac gctacatgtt	categtgege tggcttectt eggaggegtg egaceeettt	gacctgggat gccggcgaca cactggctcg gggttctcgg
20701 20761 20821	acttaagcat gcgggcccta agctggcctg cctttggctg	gagcggctcc ctttttggga cgccatcgtc gaatccgcgc caagcagatt	agcgaacaag acccacgaca aacacggccg tegegcacet tacagetteg	agctcgcggc agcgcttccc gccgcgagac gctacatgtt agtacgaggc	catcgtgcgc tggcttcctt cggaggcgtg cgaccccttt catgctgcgc	gacctgggat gccggcgaca cactggctcg gggttctcgg cgaagcgcgc
20701 20761 20821 20881	acttaagcat gcgggccta agctggcctg cctttggctg accgccggct	gagcggctcc ctttttggga cgccatcgtc gaatccgcgc caagcagatt	agcgaacaag acccacgaca aacacggccg tcgcgcacct tacagcttcg	agctcgcggc agcgcttccc gccgcgagac gctacatgtt agtacgaggc tcgagcagtc	categtgege tggetteett eggaggegtg egaceettt catgetgege cacecagace	gacctgggat gccggcgaca cactggctcg gggttctcgg cgaagcgcgc gtgcaggggc
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20701 20761 20821 20881 20941 21001 21121 21381 21361 21421 21481 21541 21601 21621 21781	acttaagcat gcgggccta agctggcctg cctttggctg accgccggct ttgcctccc ccgaccgacc tgctacaatc tcctcgcgcg cttttgataa ctggagtata gcgctggga accagtttgg agggcgcca gcgcgcgagt acactggcaa aggccgaacg tggtacact tagagggccc	gagcggctcc ctttttggga cgccatcgtc gaatccgcgc caagcagatt gcccgaccgc catggacgga catggacgga ccactcccct aatgaaacaa tgcaagttat gggccacgtt gcactgggt cactgggt cgactgccg tgcggtacac gcacgctctt gggtcatctt cgcagtgcac tgacgaggc	agcgaacaag acccacgaca aacacggccg tcgcgcacct tacagcttcg tgtctcagcc cttttttgtt aaccccacca ctgcccaccc tactttcgat ctgcgtgtat ttaaaagtcg gcggtactgg ctcggggaag gccggagatc gggttgcag gtcgctgatc gcacagctgg gggcatcagc cgtgatctgc	agctcgcggc agcgcttccc gccgcgagac gctacatgtt agtacgaggc tcgagcagtc gcatgtttt tgaacttgct tcaggcgcaa cccaccgcgc ctcaataaac aaggggttct tacttgggaa gtctcgctcc ttgaaatcac cactgggaca tgatccttgt cggcccagga atcatccccg ttgaaagctt aactggttat	categtgege tggetteett eggaggegtg egaccettt catgetgege caccagace geatgeette gaegggggtg cgcategaa ageaettat eggetegte geaettgaa acatgegeg aattgggee cattagaet caggeaeget gegegegetg egegegett geegegett getgggeett	gacctgggat gccggcgaca cactggctcg gggttctcgg cgaagcgcgc gtgcactggc ccaaacggca ctctaccgct aacgccaccg tttacatgca gttgtgcgcc ctcggggatc gctcatctgc ggtgctctgc ggtgctctgc ggtgctctgc ggcgttgctc ggcgttgctc ggcgttgctc ggcgttgctc ggcgttgctc ggcgttgctc ggcgttgctc ggcgttgctc
20701 20761 20821 20881 20941 21001 21121 21181 21241 21361 21421 21481 21541 21601 21661 21781 21781	acttaagcat gcgggccta agctggcctg cctttggctg accgccggct ttgcctccc ccgactccgc ccgaccgacc tcctcgcgcg cttttgataa ctggagtata gcgctggga accagtttgg agggcgcca gcgcgcgagt acactggcaa aggccgaacg tggtacact tagagggcc ctgaaaaaca	gagcggctcc ctttttggga cgccatcgtc gaatccgcgc caagcagatt gcccgaccgc cgctgcgga catggacgga gcaacaggtg ccactccct aatgaacaa tgcaagttat gggccacgtt gcactgggt gcactgtctt gcactgcgg tgcggtacac tgcaggtacac gcacgctctt gggtcatctt cgcagtgac	agcgaacaag acccacgaca aacacggccg tcgcgcacct tacagcttcg tgtctcagcc cttttttgtt aaccccacca ctactttcgat ctgcgtgtat ttaaaagtcg gcggtactgg ctcggggaag gccggagatc gggttgcag gtcgctgatc gcacagcag cgtgatctgg cttcccgcta	agctcgcggc agcgcttccc gccgcgagac gctacatgtt agtacgaggc tcgagcagtc gcatgtttt tgaacttgct tcaggcgcaa cccaccgcgc ctcaataaac aaggggttct tacttgggaa gtctcgctcc ttgaaatcac cactggaaca tgatccttgt cggcccagga atcatccccg ttgaaagctt tacttggtaa	categtgege tggetteett eggaggegtg egaccettt catgetgege caccagace geatgeette gaegggggtg egecategaa ageaettat egegetegte geaettgaa acatgegeg aattgggee cattagaet caggeaeget egegegeget geegegett geegegeett geegegeett teeegeaeeett	gacctgggat gccggcgaca cactggctcg gggttctcgg gggttctcgg cgaagcgcc gtgcactggc ccaaacggca ctctaccgct aacgccaccg tttacatgca gttgtgcgcc ctcggggatc gctcatctgc ggtgctctgc ggggtacttc ggggtacttc ggggtacttc ggcgttgctc ctgaggcttg catattcggg agcccctcg

FIG. 16A-6

21961	tccatctcca	ccacataata	cttgtggatc	atcaccatca	catgcagaca	cttgagctga
22021	ccctcgacat	cacaacaacc	atgateceae	agggggagg	cootocactc	ccagttctta
22021	tgcgcgatcc	cactataact	gaggatgtaa	ccttocaaca	ggcgacccat	gacggtgcta
22UBI	aatgctttct	cgccgcggcc	gaagacgcaa	agaccacaa	cctcctcatt	catccaggtc
22141	aatgetttet	gggtggtgaa	ggccagccgc	tagaccgcggg	acttateeac	atcacacaaa
22201	tggcacatct	tttggaagat	therebear	ccgggcatga	tategatege	attetecesa
22261	ccgctgtcga	cgcggtagcg	ttccatcage	acguicatgg	Latecatgee	cetececag
22321	gacgagacca	gaggcagact	cagggggttg	cgcacgttca	ggacaccggg	ggtcgcaggc
22381	tcgacgatgc	gttttccgtc	cttgccttcc	ttcaacagaa	ccggaggctg	gctgaatccc
22441	actcccacga	ttacggcatc	ttcctggggc	atctcttcgt	cggggtctac	cttggtcaca
22501	tacttaatct	ttctggcttg	cttcttttt	ggagggctgt	ccacggggac	cacgtcctcc
22561	tcggaagacc	cggagcccac	ccgctgatac	tttcggcgct	tggtgggcag	aggaggtggt
22621	aacaacaaaa	gactcctctc	ctactccagc	ggatagcgcg	ccgacccgtg	gccccggggc
22681	ggagtggcct	ctccctccat	gaaccggcgc	acgtcctgac	tgccgccggc	cattgtttcc
22741	taggggaaga	tagaggagga	gccgcgtaag	caggagcagg	aggaggactt	aaccacccac
22741	gagcaaccca	asatcaaaca	ggacctgggc	ttcgaagagc	caactcatct	agaaccccca
22001	caggatgaac	addeegagea	ggaeceggge	aaccaaaaa	agaccgacgc	tagactccag
7700T	catggctacc	aggagcacga	gcaagacgca	ctactasaac	acttocacco	ccaatccatc
22921	catggctace	Lyggaggaga	ggaggargrg	cogcoadaac	tragratura	agactatat:
22981	atcctccggg	aegeeetgge	cgaccggage	gaaacccccc	ccagcgccga	accesecac
23041	cgggcctacg	agctcaacct	cttctcgccg	egegtgeeee	ccaaacycca	gcccaacggc
23101	acctgcgagc	ccaacccgcg	tctcaacttc	tatcccgtct	ttgeggteee	egaggeeeta
23161	gccacctatc	acatctttt	caagaaccaa	aagatccccg	teteetgeeg	cgccaaccgc
23221	acccgcgccg	acgcgctcct	cgctctgggg	cccggcgcgc	gcatacctga	tatcgcttcc
23281	ctggaagagg	tgcccaagat	cttcgaaggg	ctcggtcggg	acgagacgcg	cgcggcaaac
23341	gctctgaaag	aaacagcaga	ggaagagggt	cacactagcg	ccctggtaga	gttggaaggc
23401	gacaacgcca	aactaaccat	gctcaagcgc	agcgtcgagc	tcacccactt	cgcctacccc
23461	gccgtcaacc	tecegeceaa	ggtcatgcgt	cgcatcatgg	atcagctcat	catgccccac
23521	atcgaggccc	tcgatgaaag	tcaggagcag	caccccaaga	acgcccggcc	cgtggtcagc
23581	gacgagcagc	tegegeatta	actegggace	cgcgaccccc	aggetttgga	acageggege
23501	aagctcatgc	taaccataat	cctggtcacc	ctcgageteg	aatgcatgcg	ccacttcttc
22271	agcgaccccg	agaccataca	taaggtcgag	dadaccetuc	actacacttt	caggcacggt
23701	ttcgtcaggc	agaccccgcg	catatacaaa	ataaaactaa	ccaacctaat	ctcataccta
23/01	ccegccagge	aggeetgeaa	gateceeaac	accetectec	actotactot	daadddcaad
23821	gggatcctgc	acgagaaccg	cetgggacag	accytyctcc	tetecenese	gaagggcgag
23881	gcgcgtcggg	actatgtccg	cgactgtgta	TUCCUCULA	tergecacae	ccggcaagca
23941	gccatgggcg	tgtggcagca	gtgtctcgag	gacgaaaatc	cgaaggaget	ggacaageee
24001	cttgctagaa	accttaaaaa	gctgtggacg	ggcttcgacg	agegeacege	egeeteggae
24061	ctggccgaga	tegtttttcc	agaacgcctg	aggcagacgc	tgaaaggcgg	getgeeegae
24121	ttcatgagcc	agagcatgtt	gcaaaactac	cgcactttca	ttctcgagcg	acctgggatg
24181	ctacccgcca	cctgcaacgc	attcccctcc	gactttgtcc	cgctgagcta	ccgcgagtgt
24241	ccccaccac	tgtggagcca	ctgctatctc	ttgcagctgg	ccaactacat	cgcctaccac
24301	tcggacgtga	tcgaggacgt	gagcggcgag	gggcttctcg	agtgccactg	ccgctgcaac
24361	ctgtgctccc	cacaccactc	cctggtctgc	aacccccagc	ttctgagcga	gacccaggtc
24421	atcggtacct	tegagetgea	aggtccgcag	gagtccaccg	ctccgctgaa	actcacgccg
24481	gggttgtgga	cttcccccta	cctgcgcaaa	tttgtacccg	aggactacca	cgcccatgaa
2/5/1	ataaagttct	trangarra	atcococcca	cagcacgcgg	atctcacccc	ctgcgtcatc
24241	acccagggcg	castectere	ccaattgcac	accatecaaa	aatcccccca	agagtttctt
24001	ctaaaaaagg	atagagaat	ctacctggac	accesacea	acasaatact	caacccgggt
24001	ctaaaaaagg	gragaggggr	craceagae	accarateata	gegaggegee	aanaanaatn
24/21	ctccccagc	acgeegagga	agaagtagga	geegeeageg	gagcagacgg	aattogaaca
24/81	ggacagccag	gcagaggagg	acyaacygya	ggaggagaca	gaggaggaag	caacagaaga
24841	ggtggaagag	gagcaggaaa	cagageagee	egregeegea	ccatecgege	cygcaycccc
24901	gccggtcacg	gatacaacct	ccacagetee	ggccaagcct	cetegragat	gggaccgagc
24961	gaagggtgac	ggtaagcacg	agcggcaggg	ctaccgatca	rggagggtcc	acaaagccgc
25021	gatcatcgcc	tgcttgcaag	actgcggggg	gaacatcgct	ttegeeegee	gctacctgct
25081	cttccaccgc	ggggtgaaca	tcccccgcaa	cgtgttgcat	tactaccgtc	accttcacag
25141	ctaagaaaaa	gcaagtaaga	ggagtcgccg	gaggaggcct	gaggatcgcg	gcgaacgagc
25201	cctcgaccac	cagggagctg	aggaaccgga	tcttccccac	tctttatgcc	atttttcagc
25261	agagtcgagg	tcagcagcaa	gaactgaaag	taaaaaaccg	gtctctgcgc	tcgctcaccc
25321	gcagttgctt	gtaccacaaa	aacgaagatc	agctgcagcg	cactctcgaa	gacgccgagg
25381	ctctgttcca	caagtactec	gcgctcactc	ttaaagacta	aggcgcqccc	acccggaaaa
25441	aaggcgggaa	ttacctcatc	gccaccatga	gcaaggagat	tcccacccct	tacatotoga
25501	gctatcagcc	ccadatadac	ctaaccacaa	acaceteeca	ggactactcc	acccocatoa
25561	actggctcag	taccaacca	togatgatch	cacqqqtcaa	cagaatccat	aaccatcgaa
-220H	acoggettag	-g-cggcccc			-3355-0050	

FIG. 16A-7

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25621	accagatatt	gttggagcag	gcggcggtca	cctccacgcc	cagggcaaag	ctcaacccgc
	gtaattggcc					
25741	cgcgtgacgc	200000000	atacaaataa	atasatasaa	tataanaata	3000000000
	cttcccggtg					
25861	gcacacagct	caacgacgag	ttggtgagct	cttcgatcgg	tctgcgaccg	gacggagtgt
25921	tccaactagc	caasaccaaa	agategtect	tcactcccaa	ccaggcctac	ctgaccttgc
	agagcagctc					
26041	ttgtgccctc	ggtctacttc	aaccccttct	cgggatcgcc	aggcctctac	ccggacgagt
26101	ttataccgaa	cttcgacgca	gtgagagaag	cggtggacgg	ctacgactga	atgtcccatg
26161	gtgactcggc	tgaggtcggt	coattgagge	atctggacca	ctaccaccac	ctacactact
	tcgcccggga					
	ctgcacacgg					
26341	tcacccagca	accetteetg	gtcgagcggg	accggggagc	taccacctac	accgtctact
26401	gcatctgtcc	taccccgaag	ttgcatgaga	atttttgctg	tactctttgt	ggtgagttta
	ataaaagctg					
		_		_		_
	atcaacttca					
	atctggtttt					
26641	ctcctaccta	acaatctcac	cagtggacta	accttctcag	ttaaaagggc	aaagctaatt
26701	cttcatcgcc	ctattotaga	aggaacttac	cagtgtcaga	acagacctta	cttccacagt
	ttcactttgg					
	tctgatacta					
	gggagttcta					
26941	gcagtgctgt	atcaacttcc	ttgctgggtc	gaaatcaggg	tatttatctg	ctgggtcaga
	cattgtgggg					
	tgctgtcatg					
	gctctgtagt					
27181	ccatgggaaa	tgtatgggtg	ggattctggc	aaccaggaga	tgagcagaac	tacacggtca
27241	ctgtccatgg	tagcgatggc	aatcacactt	tcggtttcaa	attcattttt	gaagtcatgt
	gtgatatcac					
	tggtgggttt					
	gggctctagt					
27481	tgctataaat	tctttttctc	ttcgcacaac	catgaataca	gtgttccgta	tcgtgctgct
27541	ctctcttctt	gtagctttcg	otcaggcagg	aattcatatt	attaatocta	catagtagga
	taatataact					
	gcaattttgt					
27721	aaacttaact	ctgattcatg	ttaacaaaac	ccatgaaaga	acatacatgg	gttacagaca
27781	tgacagtaag	ggaaaagtag	actataaggt	tacagtcatt	ccacctcctc	ctgctactgt
	aaagccacaa					
	tggacctcca					
	agataaaatt					
28021	gtttgtaaac	tttacacatg	atggaggcta	tcttggattc	aattacaaag	gtactcagag
28081	aattcagtat	gaggttatag	ttttagatcg	atttccaaat	tctggtcaga	tgaaaattga
	agaacaaagt					-
	gggtatagat					
	aagaagacgg					
28321	cttaacttta	gttggtccag	atggaaaagt	cacttggtat	gatggtgatt	taaaaagacc
28381	atgtgaagaa	caaaactata	ggcttccaca	tcagtgtagt	gctcagaact	taactttaat
	taatgtaact					
	aagatacaga					
	cagacctact					
28621	tgatgaagaa	tcaaaaattc	catctactac	tgtggcaatc	gtggtgggag	tgattgcggg
28681	cttcataact	ataatcatto	tcattctgtg	ctacatctgc	tgccgcaagc	gtcccagggc
	atacaatcat					
	ttcagaacca					
	gctgcaaatg					
28921	gctggaatta	atactacatg	gacagggtat	tttaatgagg	gtccaaaagg	aaaaaatggg
	tggatgaata					
	attactaatc					
	gaaagtttta					
29161	attattgagg	ttccaacaac	tagagcaccc	accacagtta	ggacaacaca	gcctaccact
	gtgcccacta					

FIG. 16A-8

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00001		acacacact	gcagaatact	actttattga	ttgggttttt	actgagagga
00044		ataataaaa	ascadadct.	ACCECAAULU	CCLLCaucau	Caccycaaac
29341	aatgaaagta	thactgaaca	taatgaaaacc	ggagtatcat	tgatgaatcg	acageettae
29401	ttaacttcgc	ttgettggae	tacttttctc	attatetata	ggatctttat	tettacaatt
29461	tcaggtttgg	atattcaaat	cacciccic	gacgactgtg	aacaacccat	atacaggcca
29521	cttctgtact	ttgtctgctg	caaayccaya	gagaaaccca	ggcggcccat	tetettetet
29581	gtaatcgggg	aacctcagcc	tetecaagty	gatggagget	taaggaatct	cctattctat
29,641	tttacagtat	ggtgatcagc	catgattcct	aggttettee	tatttaacat	atatataaaa
20701	atattassas	tetatactac	cttcacaacc	gtctcgcacg	Cologodoga	Ctgtctaggg
20761	catttcccaa	catacctcct	ctttacccta	ctaacctgca	cctycytcty	Caycaccycc
		+	acaactcatc	dactddtdct	acacacacta	Caattatte
00001		cccaatacac	dascasass	gragecagaa	tettaagget	Cattlyatta
00041		MATCS TACEA	ararcccrcc	Laticicity	CCCGCCacc	
20001		resetterer	gacatatgga.	atttcttaga	ttgctattag	yayaaaaccy
70061		ctattactta	Granteatta	dudLauttat	gguctgucu	cgcacoco
20121	++~acs++s+	matchacccc.	tottttaatc	ttggctggaa	Cicigulyay	gcattcacat
30121	Legecateat	azacacttca	ctagecteca	caccaccacc	cacaccgcct	ccccgcagaa
30181	acacactaga	tateatteae	tacttacaac	ageccetee	ceggeceet	tccactgtta
30241	accagtteee	catgatteag	cacciagaag	gaccacctgg	acctcgagat	agacagccag
30301	gctactttca	cataaccggc	ggcgatgact	atccaectss	agcaggagcg	ggccgccaag
30361	gcctccgagc	agegeateet	geaactgege	bassassas	acetattata	cctaatcaag
30421	gageteeteg	atgccatcaa	catceaceag	tycaayaayy	gcatcttctg	cacctataaa
30481	caggcaaaga	tcacctacga	getegtee	ggeggeaage	agcatcgcct	agtcatcacc
30541	ctaccccagc	agaagcaaaa	gttcacctgc	atggtgggcg	tcaaccccat	agicaccacc
20601	anagart caa	acasasccss.	caactacatc	cactoctcct	gegaaageee	cgagtgtatt
20661	tactacatac	traaracect	ttacaaactc	cacaacctcc	Lececatgaa	ctgatgttga
20721	*********	aaaaaccaat	caaacccttc	cccaattact	Cataayaata.	aaccaccyga
20701	agtaatgatt	caataaadat	cacttacttq	aaatctqaaa	gtatgtetet	ggcgcagccg
20041	ht	cctcccaacc	ctdctcccag	ctctggtact	ccagtccccg	gegggeggeg
20001	anattactac	acaccttdaa	agggatgtca	aattcctggt	ccacaattt	Callylllic
20061		casararret	ccaaataaaa	gatgacttca	accccgtcta	CCCCtatggc
21021	+	atcacaatat	ccccttcctt	actccccct	ELGELLCLU	cyatygatte
31021	cacgegegga	cacatacaat	cctateacte	aaactggctg	acccaatcgc	catcactaat
31081	Cadadctttt	caccegggge	addagggggg	cttactgttg	aaaaagatag	tggaaatcta
31141	ggggatgttt	cactcaaggt	attaceaatt	acaactgata	aacagttgga	aattgcactg
31201	aaggtgaacc	ttaaggttet	taateeeae	cttoocataa	aagcaggtca	tagattgaaa
31261	gcttatccat	ttgaagtcag	thategeaag	ttaaceaata	cacttataat	tttgactgga
31321	gtcattgaca	aaattgctgg	tetgyaagge	ceggeaggea	cgcttgtagt	agttggtata
31381	aaaggaatag	gtactgaaaa	tettgaaaac	agtgatgggt	caagtagagg	tttaattact
31441	aacgtaagac	ttgctaaaga	tggaggtctg	tettttgata	aaaagggtga	accesettat
31501	tggaataaac	atgatgacag	acgcactcta	tggacaacte	ccgacccatc	terasatass
21561	agaatcgatc	addaaaddda	ttcaaagctc	actitagiai	taataaaaty	tygtagttaa
21621	attttaacta	atototott	acttottota	aaaggaaaat	ttagtaacat	aaacaacaac
21601	antastorsa	ctcataaaaa	aatcacaqta	aagctacttt	ttaatgaaaa	gyyaytatta
21741	-terrerett	ccacacttaa	gaaagaatat	togaactaca	gaaatgataa	Licialigia
21001	tatasaacet	atgataatgc	agttcctttt	atgccaaaca	taaaagctta	Lectaaacet
21061	accacadaca	cttcggctaa	accagaagat	aaaaaaagtg	Ctycladady	atacattyty
21021	aggaatetet	atattanaaa	cttgccagat	aaaactgttg	ttataactat	Laayiilaai
21001	acadaaacto	. aatgtgctta	ttcgattacc	tttgaattca	. catgggcaaa	aaccucugaa
22041	- ~- tatacaat	ttgattcctc	ctcttttacc	ttttcctata	ttgcccaaga	aaatyayyat
22101	- maamamaaat	· aaaatotttt	aaaatqaatt	catgtatett	tattgattt	Lacaccayca
22101	. gaagacaaa	atctcccacc	accagcccat	ttcacagtgt	aaacgattct	ctcagcacgg
22727	. cyggtagtta	, gtctcccact	gttctgatta	atacaggaac	tggacttggg	gtctataatc
32221	. gtggccttaa	acayyyaaac	geeeggaaaa	tcggtgatto	agatgaagcc	gtcctctgaa
32281	. cacacaguu	. cctggcgagc	acactecaan	gtcacagtct	ggtgaaacga	gaagaacgca
32341	aagtcatcca	agegggeete	acaytecaag	tatacctctc	catcagege	ctcaacagtc
32401	. cagattcata	ctcggaaaac	aggatgggtt	cgcgccccc	catcagegee	atetetetaa
32461	tetgeegeeg	gggctcggtg	eggergerge	ayaryyyart	gggatcacaa	caccccatcc
コクドウェ	- atatastaca	· cacagootto	agcatcagto	tcctagtaca	rtegggeacag	cacegeatice
22501	t east ct cact	· catottotoa	cagtaagtgc	: agcacataat	: caccatgtta	tteageagee
22641	l astasttcac	, aatactccao	ccaaaactca	l tattggggat	: gatggaaccc	acgigaccai
22701	l cataccadat	· acaacaatat	atcagatgcc	: tgcccctcat	: gaacacactg	CCCatataca
22761	Leatetett	gaggatatet	: ctottcacaa	ı tctgacggta	l ccagggaaag	cgctggttga
22021	Lacatoracco	· gtaaatgact	: ctcctgaacc	: acacggccad	r cagggtgcct	eccycecyac
3288	actocaoooa	acccaaaaat	gaacagtggc	aatgcaggat	ccagcgctcg	tacccgctca
J200.		. 39000				

FIG. 16A-9

33001	ttaaaatttt	tctcaccaag tatttcctct	ggagtcaaga	tcatatccca	ggggactgga	aactcttgga
33061	gcagggtaaa	gccagcagca	catggtaatc	cacggacaga	acttacatta	tgataatctg
33121	catgatcaca	atcaggcaac	aggggatgtt	gttcagtcag	tgaagccctg	gtttcctcat
33181	cagatcgtgg	taaacgggcc	ctgcgatatg	gatgatggcg	gagcgagctg	gattgaatct
33241	cogtttgcat	totagtggat	tctcttgcgt	accttgtcgt	acttctgcca	gcagaaatgg
33301	gcccttgaac	agcagatacc	cctcctgcgg	ccgtcctttc	gctgctgccg	ctcagtcatc
33361	caactgaagt	acatccattc	tcgaagattc	tggagaagtt	cctctgcatc	tgatgaaaca
33421	aaaaacccgt	ccatgcgaat	tcccctcatc	acatcagcca	ggactctgta	ggccatcccc
33481	atccagttaa	tgctgccttg	tctatcattc	agaggggggg	gtggcaggat	tggaagaacc
33541	atttttattc	caaacggtct	cgaaggacġa	taaagtgcaa	gtcacgcagg	tgacagcgtt
33601	ccctccact	atactaataa	aaacagacag	ccaggtcaaa	acccactcta	ttttcaaggt
33661	actcaaccat	ggcttcgagc	agtggctcta	cgcgtacatc	cagcataaga	atcacattaa
33721	aggetggeee	tccatcgatt	tcatcaatca	tcaggttaca	ttcctgcacc	atccccaggt
33781	aattctcatt	tttccagcct	tggattatct	ctacaaattg	ttggtgtaag	tccactccgc
33841	acatotogaa	aagctcccac	agtgccccct	ccactttcat	aatcaggcag	accttcataa
33901	tagaaacaga	tectactact	ccaccacctg	cagcgtgttc	aaaacaacaa	gattcaataa
33961	gattctaccc	teegeeetga	gctcgcgcct	caatgtcagc	tgcaaaaaat	cacttaagtc
34021	ctgggccact	acagctgaca	attcagagcc	agggctaagc	gtgggactgg	caagcgtaag
34081	ggaaaacttt	aatgctccaa	agctagcacc	caaaaactgc	atgctggaat	aagctctctt
34141	tatatctccg	gtgatgcctt	ccaaaatgtg	agtgataaag	cgtggtagtt	tttctttaat
34201	catttgcgta	atagaaaagt	cctgtaaata	agtcactagg	accccaggga	ccacaatgtg
34261	otaocttaca	ccacatcact	gaagcatggt	tagtagagat	gagagtctga	aaaacagaaa
34321	gcatgcacta	aactaaggtg	gctattttca	ctgaaggaaa	aatcactctc	tccaacaaca
34381	gggtacccac	tagatagacca	ttgcggacat	acaaaaatcg	gtccgtgtga	ttaaaaagca
34441	gcacagtaag	ttcctgtctt	cttccggcaa	aaatcacatc	ggactgggtt	agtatgtccc
34501	tggcatggta	gtcattcaag	gccataaatc	tgccctgata	tccagtagga	accagcacac
34561	tcacttttag	gtgaagcaat	accaccccat	gcggaggaat	gtggaaagat	tcagggcaaa
34621	aaaaattata	tctattgcta	gtcccttcct	ggacgggagc	aatccctcca	ggactatcta
34681	tgaaagcata	cagagattca	gccatagctc	agcccgctta	ccagtagaca	gagagcacag
34741	cagtacaagc	gccaacagca	gcgactgact	acccactgac	ccagctccct	atttaaaggc
34801	gccttacact	gacgtaatga	ccaaaggtct	aaaaaccccg	ccaaaaaaaa	acacacacgc
34861	cctgggtgtt	ttttgcgaaa	acacttccgc	gttctcactt	cctcgtattg	atttcgtgac
34921	ttaacttccg	ggttcccacg	ttacgtcact	tctgccctta	catgtaactc	agtcgtaggg
34981	caccatctta	cccacgtcca	aaatggcttc	catgtccagc	cacgcctccg	cggcgaccgt
35041	tagccgtgcg	tcgtgacgtc	atttgcatca	tcttctctcg	tccaatcagc	gctggccccg
35101	ccctaaattc	aaaagctcat	ttgcatgtta	acttttgttt	actttgtggg	gtatattatt
	gatgatc					
SEQ I	D NO: 5		•			

Grp	Vaccine	Monkey	Р	re	W	k 4	W	k 8	W	12_
•	at Wk 0, Wk 4	, ID	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
1	Ad24AE 1gcgAOrf8Ad5Orf6	00C072	3	4	4	381	3	150	3	68
	10^11 vp	00C178	3	3	1	559	1	743	0	635
	·	00C222	0	3	1	369	1	753	0	670
		00D011	1	9	9	211	4	273	0	520
•		00D023	٥	6	0	295	1	459	1	368
		00D031	15	5	10	103	1	101	1	40
2	Ad24AE 1gogAOrf6Ad5Orf6	99C168	4	6	0	118	5	241	3	209
	10^10 vp	99C170	10	5	5	241	3	141	3	103
		99C173	1	3	l °	23	0	14	0	21
3	Ad24AE 1gogAE4Ad5Orf6	99C154	0	3	0	93	0	60	1	53
	10^10 vp	99C158	1	0	1	141	0	101	1	120
		99C177	0	0	٥	45	0	39	0	79
4	MRKAd5-HIVgag	00C018	1	5	13	1025	0	824	3	753
	10^11 vp	00C034	0	4	5	219	5	404	0	491
		00C058	4	4	3	1086	0	440	0	439
5	MRKAd5-HIVgag	99C218	0	3	5	2500	0	1580	10	1655
•	10^10 vp	99C227	6	1	4	529	5	365	5	1004
		99D185	ND	ND	0	425	0	310	0	271

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Vaccine	Monkey	Gag-Specific (Wk 1:	
at Wk 0, Wk 4	ID	%CD4	%CD8
Ad24AE 1 gagAOrf6Ad5Orf6	00C072	0.02	0.02
10^11 vp	00C178	0.05	0.38
	00C222	0.02	0.40
	00D011	0.02	0.27
	00D023	0.01	0.11
	00D031	0.01	0.01
MRKAd5-HIVgag	00C018	0.05	0.41
10^11 vp	00C034	0.06	0.18
•	00C058	0.02	0.28

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Grp	Vaccine	Monkey	Wk 4	WK 8
	at Wk 0, Wk 4	ID		
1	Ad24ΔE 1 gagΔOrf6Ad5Orf6	00C072	<10	77
	10^11 vp	00C178	<10	26
	·	00C222	<10	423.
		00D011	<10	98
		00D023	<10	<10
		00D031	<10	<10
	A 404457 A 040445040	000100	10	- 10
2	Ad24∆E 1gog∆Orf6Ad5Orf6	99C168	<10	<10
j	10^10 vp	99C170	<10	<10
	·	99C173	<10	<10
3	Ad24ΔE 1 gagΔE4Ad5Orf6	99C154	<10	<10
	10^10 vp	99C158	<10	<10
		99C177	<10	<10
4	MRKAd5-HIVgag	00C018	34	1017
	10^11 vp	00C034	14	423
	·	00C058	46	934
	APICA JE LIVERA	000010	00	- 00
5	MRKAd5-HIVgag	99C218	20	99
	10^10 vp	99C227	40	767
		99D185	17	342

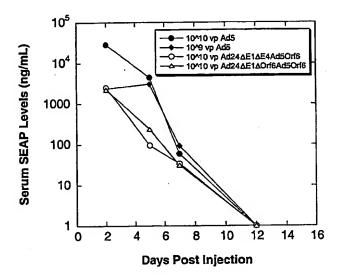


FIG. 20

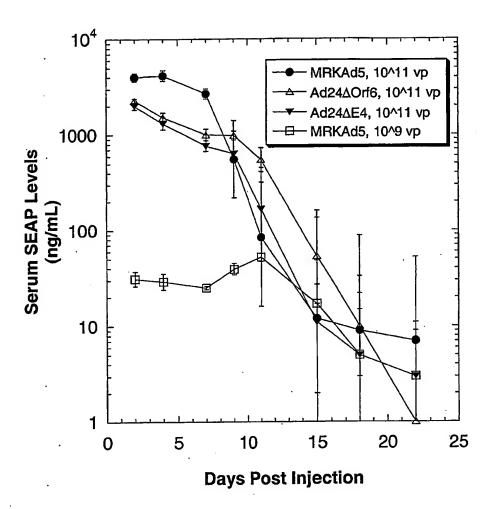
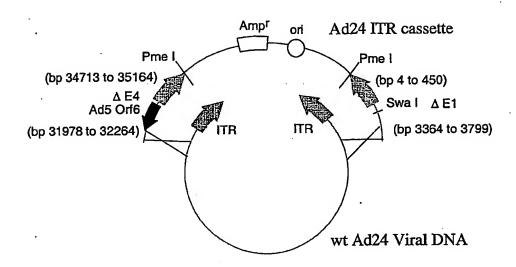


FIG. 21

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Animai	Prime (Wk 0, 4, 26)	Boost (Wk 56)	Pre		Prime ^b		Pre-Boost ^e		Post-Boost ^d	
At manage			Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
Monkey 1	10° vp MRKAd5-gag	1011 vp Ad24ΔE1gagΔOrf6Ad5Orf6	18	16	1	244	3	74	3	1235
Monkey 2	107 vp MRKAd5-gag	1011 vp Ad24AE1gagAOrf6Ad5Orf8	10	9	4	83	0	18	٥	856
Monkey 3	10° vp MRKAd8-gag	1011 vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	1	0	219	9	69	0	703
Monkey 4	10 ⁷ vp MRKAd8-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf8	1	1	3	59	1	20	0	419
Mankey 5	none	10 ¹¹ vp Ad24AE1gagAOrf6Ad5Orf8	3	4	ND*	ND	ND	ND	4	558
Monkey 6	none	1011 vp Ad24AE1gagAOrf6Ad5Orf6	0	3	ND	ND	ND	ND	1	295
Monkey 7	none	1011 vp Ad24AE1gagAOrf6Ad5Orf6	1	8	ND	ND	ND	ND	8	103 *
Monkey 8	none	1011 vp Ad24AE1gagAOrf6Ad5Orf6	3	3	ND	ND	ND	ND	1	381
Monkey 9	none	1011 vp Ad24AE1gagAOrf6Ad5Orf6	1 0	6	ND	ND	ND	ND	0	369
Monkey 10	попе	1011 vp Ad24AE1gagAOrf6Ad5Orf6	15	5	ND	ND	ND	ND_	10	211

Animal	Prime (Wk 0, 4, 26)	Boost (Wk 56)	Gag-Specific T cells (Wk 60)		
Ailillai	t time (vik of 4) 20)		%CD4	%CD8	
Monkey 1	109 vp MRKAd5-gag	10 ¹¹ vp Ad24∆E1gag∆Orf6Ad5Orf6	0.06	0.37	
Monkey 2	107 vp MRKAd5-gag	10 ¹¹ vp Ad24∆E1gag∆Orf6Ad5Orf6	0.01	0.56	
Monkey 3	109 vp MRKAd6-gag	10 ¹¹ vp Ad24∆E1gag∆Orf6Ad5Orf6	0.07	0.06	
Monkey 4	10 ⁷ vp MRKAd6-gag	10 ¹¹ vp Ad24∆E1gag∆Orf6Ad5Orf6	0.04	0.20	

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Animal	Prime (Wk 0, 4)	Boost (Wk 24)	Pre		Prime ^b		Pre-Boost*		Post-Boost ^d	
			Mock	Gag*	Mock	Gag	Mock	Gag	Mock	Gag
Monkey 11	1011 vp Ad24AE1gagAOrf6Ad5Orf6	107 vp MRKAd5-gag	3	4	3	150	4	28	0	188
Monkey 12	1011 vp Ad24AE1gagAOrf8Ad5Orf6	107 vp MRKAd5-gag	0	3	1	753	4	554	0	1029
Monkey 13	1011 vp Ad24ΔE1gagΔOrl6Ad5Orl8	10 ⁷ vp MRKAd5-gag	1	9	4	273	0	370	0	1520
Monkey 14	none	10 ⁷ vp MRKAd5-gag	0	0	ND*	ND	ND	ND	4	94
Monkey 15	none	107 vp MRKAd5-gag	0	0	ND .	ND	ND	ND	1	168
Monkey 16	none	107 vp MRKAd5-gag	8	3	_ND	ND	_ND	ND	_8	149

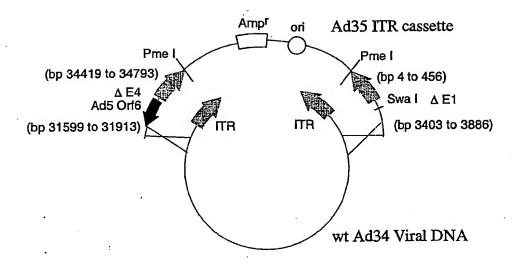
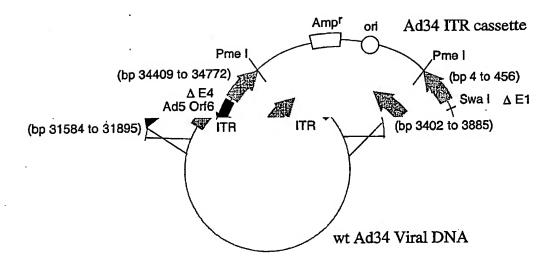


FIG. 26



1	catcatcaat	aatatacctt	atagatggaa	tootoccaat	atgtaaatga	ggtgatttta
61	aaaattataa	aatatataat	gattggctgt	aggattaagg	actonocac	9909=====
101	addittgtgg	ggcgcgcgc	gactggctgt	ggggctaacg	yctaaacyyy	acaacacaac
121	cgtgggaaaa	tgacgttttg	tgggggtgga	gtttttttgc	aagttgtcgc	gggaaatgtg
181	acccataaaa	aggetttttt	tctcacggaa	ctactgactt	ttcccacaat	atttaacagg
2/1	aaatgaggta	attttaacca	gatgcaagtg	aaaattaata	atttacagga	assasatass
.241	aaacyayyca	gruitgatty	gargeaageg	addattytty	actigegege	gaaaactgaa
301	tgaggaagtg	tttttctgaa	taatgtggta	tttatggcag	ggtggagtat	ttgttcaggg
361	ccaggtagac	tttgacccat	tacgtggagg	tttcgattac	catatttttt	acctgaattt
421	coccetacee	tatazzzata	++++++++	200030000		
421	ccgcgcaccg	Lyccaaaycc	ttctgttttt	acgraggige	cagergareg	ctacggtatt
481	tatacctcag	ggtttgtgtc	aagaggccac	tcttgagtgc	cagcgagaag	agttttctcc
541	tetacaccaa	cagtttaata	ataaaaaaat	gagagatttg	coatttctoc	ctcaggaaat
601	aatttataat	asasataasa	atgaaataat	3-3-3-6-6		testesses
001	aatttetget	yayactyyaa	atgaaatact	ggagerigig	gracacacac	cgacgggaga
661	cgatccggag	ccacctgtgc	agctttttga	gcctcctacg	cttcaggaac	tgtatgattt
721	agaggtagag	ggatcggagg	attctaatga	ggaagetgtg	aatggctttt	ttaccgattc
791	tateettta	actactacta	naganthaga	35500505	acttte	chthasatas
761	Latycutta	googecaacg	aaggattaga	actagateeg	cctttggaca	ctttegatae
841	tccaggggtg	attgtggaaa	gcggtacagg	tgtaagaaaa	ttacctgatt	tgggttccgt
901	ggactgtgat	ttgcactgct	atgaagacgg	atttcctcca	agtgatgagg	aggaccatga
961	asaddadcad	totatocana	ctgcagcggg	tasaaasata	-3-5-5-55	atattaatt
1001	adaggagcag		Cogcagoggg	Lyayyyayty	aayyetyeea	graceagere
1021	tcagttggat	tgcccggagc	ttcctggaca	tggctgtaag	tcttgtgaat	ttcacaggaa
1081	aaatactgga	gtaaaggaac	tgttatgttc	octttottat	atgagagggg	actoccactt
1141	tatttacagt	aantntnttt	aagttaaaat	ttaaaggaat	2+44+4++	tengatetat
1001	catttacagt	auguguguu	aagttaaaat	ccaaaggaac	acyclyclic	ccacatgtat
1201	attgagtggg	agttttgtgc	ttcttattat	aggtcctgtg	tctgatgctg	atgagtcacc
1261	atctcctgat	tctactacct	cacctcctga	gattcaagca	cctattccta	tagacataca
1321	caacccatt	cctatasaa	ttaagcctgg	gaaaggtgga	aasataass	2225
1301	caageceaee	cctgtgaagt	ccaagecegg	gaaacgicca	ycaycyyaaa	aacttgagga
T28T	cttgttacag	ggrggggacg	gacctttgga	cttgagtaca	cggaaacggc	caagacaata
1441	agtgttccat	atccgtgttt	acttaaggtg	acotcaatat	ttgtgtgaga	gtgcaatgta
1501	ataaaaatat	attaactatt	cactggtttt	tattoctttt	tagacagaga	ctcacctata
1561		gecuacige	Lactygette	caccycccc	cygycygya	cicaggiata
TOOT	taagtagaag	cagacctgta	tggttagctc	ataggagctg	gctttcatcc	atggaggttt
1621	gggccatttt	ggaagacctt	agaaagacta	ggcaactgtt	agaggacgct	teggaeggag
1681	tetecaattt	ttggagattc	tggttcgcta	gtgaattage	tagggtagtt	tttaggataa
1741	2262665	*********	tttesses	b-bbb-	therene	-t-t-t-t-
1/41	aacayyacca	Laaayaayaa	tttgaaaagt	tgttggtaga	ttgcccagga	ctttttgaag
1801	ctcttaattt	gggccatcaa	gttcacttta	aagaaaaagt	tttatcagtt	ttagactttt
1861	caaccccagg	tagaactgcc	gctgctgtgg	cttttcttac	ttttatatta	gataaatgga
1021	taccacacac	tastttasaa	3003003033	****		gacaaacgga
1921	ccccycagac	ccaccicage.	aggggatacg	LLLLygalll	cytayccaca	gcarrgraga
1981	gaacatggaa	ggttcgcaag	atgaggacaa	tcttaggtta	ctggccagtg	cagcctttgg
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<b>5</b> 191	gtctcctgaa	ctgcaacggg	tgcttactgg	atctacgtcc	actggacggg	ataggggcgt
2221	taagagggag	agggcatcta	gtggtactga	tgctagatct	gagttggctt	taagtttaat
2281	gagtcgcaga	catactasss	ccatttggtg	acetaeaata	Cadasadadd	gaagggatga
2241		********		godogogoc	cagaaagagg	gaagggacga
2341	aytttctgta	ccgcaggaga	aatattcact	ggaacaggtg	aaaacatgtt	ggttggagcc
2401	tgaggatgat	tgggaggtgg	ccattaaaaa	ttatgccaag	atagctttga	ggcctgataa
2461	acagtataag	attactagac	ggattaatat	ccanaatact	tattacatat	ctagaaatag
2521	aastanaata	ataataaata	2522222	coggaacgcc		
2321	ggergaggrg	giaalagala				
2581	ataacctaaa		CCCaagacaa	ggcagttatt	agatgctgca	tgatggatat
2641	2-22-0-23-	gtagtcggta	tggaagcagt	aacttttgta	agatgctgca aatgttaagt	tgatggatat ttaggggaga
2701	togttataat	gtagtcggta	tggaagcagt	aacttttgta	agatgctgca aatgttaagt	tgatggatat ttaggggaga
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2821 2881 3001 3121 3181 3241 3361 3421 3481 3561 3661	tggttataat ttttggtttc tttctatgcg atgcatattc ctgcgcttct taacagcttat gcattgtaat ttttgatcac tatgccttac cagaatgagc tgatgatacg gccggtgtgt tggagcagag ggggtggggt	gtagtcggta ggaatagtgt aacaatacct tgttggattg caaagatgta acagatactg tgcgggtgctt atgctggcta aatgtgttga cagtgtaaca ctaacaggaa agatcgaggg gtagatgtga ttcggatcca tttcagatgg aaacgcttct ggcaggagtt caattcttca cgccgccgcc	tggaagcagt ttatggccaa gtgtagatgc ccacagctgg acctgggcat gatgttat ccgatgagat ccaagtgtac tgaatcatgt tctttgacat tctttgacat gtggagaaga ctgaagatct gtggagaaga ccgaagatg cgtcagaacg cgtcagaacg	aacttttgta taccaaactt ctggggacag cagaaccaag tctgaatgaa tttaattaag gccttatcaa tgtttcccat catgcatgca gaaagtgttg gaacatgcaa cgaatgcgga gagaccggat aactgactaa taaaaatttg gagtcttcag ttatgggat catgctactt	agatgctgca aatgttaagt atattgcatg gttagtgtac agtcaattgt ggcgaagcaa ggcaatgcca atgctcactt caacgcaaaa ggtgggcgta ttggaaccag atctggaaga ggcaagcatg catttggtta ggtgagtatt tttttctgt cccttatctg tacgtgat aagttctca taggttaa	tgatggatat ttaggggaga gttgtagctt ggggatgtag ctctgaagaa gggtccgcca gcgtaaagca gtgccggtgg aatggcctgt gaggaatgtt atgccttttc tcctgaggta ccaggttcca ttgcccgcac gggaaaactt ctttcagctg acagggcgtc gcgtagacgccg acagggcgtc
2821 2881 3001 30121 3181 3241 3361 3421 3541 3661 3661 3721	tggttataat ttttggtttc tttctatgcg atgcatattc ctgcgcttct taacagttgtaat ttttgatcac tatgccttac cagaatgagc tgatgatacg gccggtgtgt tggagcagag ggggtggggt	gtagtcggta ggaatagtgt aacaatacct tgttggattg caaagatgta acagatactg tgcggtgctt atgctggcta aatgtgttga cagtgtaaca ctaacaggaa agatcgaggg gtagatgtga ttcggatcca tttcagatgg aaacgcttct ggcaggagtt cagtgtcacc cgccgcc cgtggctaat	tggaagcagt ttatggccaa gtgtagatgc ccacagctgg acctgggcat gatgtttat ccgatgagag ctgatgtac tgaatcatgt tctttgacat tctttgacat gtggagaaga tttaagggg ctgaagatct gtggagaaga ctgaagatct gtgtagaatct gtgtagaatct gtcagaatct gtcagaatct tctaaggag tctaaggact tctaagaatct tctacttct	aacttttgta taccaaactt ctggggacag cagaaccaag tctgaatgaa tttaattaag gccttatcaa tgtttcccat catgcatgca gaaagtgttg gaacatgcaa cgaatgcgga gagaccggat aactgactaa taaaaatttg gagtcttcag ttatggatc atgcatca ccctaataacc	agatgctgca aatgttaagt atattgcatg gttagtgtac agtcaattgt ggcgaagcaa ggcaatgcca atgctcactt caacgcaaaa ggtgggcgta ttggaaccag atctggaagcatg catttggta ggtagtatt tttttctgt cccttatctg tactgtggat aagtcttca tgtgtggttggat atgtgttgat tttttctgt tccttatctg tactgtggat atgtgttgat tttttcatgt tccttaccgt	tgatggatat ttaggggaga gttgtagctt ggggatgtag ctctgaagaa gggtccgcca gcgtaaagca gtgccggtgg aatggcctgt gaggaatgtt atgcctttc tcctgaggta ccaggtcca gggaaactt ctttcagctg acagggcgtc ggaagacccg cctgtggacg atggcaca acggcgtc
2821 2881 2941 3001 3012 3181 3241 3361 3421 3561 3601 3721 3781	tggttataat ttttggtttc tttctatgcg atgcatattc ctgcgcttct taacattgcatt gcattgtaat ttttgatcac tatgccttac cagaatgagc tgatgatacg gccggtgtgt tggagcagag ggggtggggt	gtagtcggta ggaatagtgt aacaatacct tgttggattg caaagatgta acagatactg tgcggtgctt atgctggcta aatgtgttga cagtgtaaca ctaacagaga gtagatgtga ttcggatcca ttcagatgg aaacgcttct ggcaggagtt caattctca cgtggctaat cgtggctaat	tggaagcagt ttatggccaa gtgtagatgg ccacagctgg acttgggcat gatgtttat ccgatgagag ctgtgcatat ccaagtgtac tgaatcatgt tctttgacat tctttgacat tcggagaaga ctgtgaagatga acagattgag tttaaggggg tttaaggggg tctaagaattg cgtcagaact gcgctctgacct gcctcttct tcagctgaq	aacttttgta taccaaactt ctggggacag cagaaccaag tctgaatgaa tttaattaag gccttatcaa tgtttcccat catgcatgca gaaagtgttg gaacatgcga gagaccggat aactgcgat aactgactaa taaaaatttg gagtcttcag ttatgggatc atgctacttt ccgctaacac ctataaccc ctttgaccca	agatgctgca aatgttaagt atattgcatg gttagtgtac agtcaattgt ggcgaagcaa ggcaatgcca atgctcactt caacgcaaaa ggtgggcgta ttggaaccag atctggaacatg catttggta ggtaggaatt tttttctgt cccttatctg tactgtggat agttcttca tgtgcttggat agttcttca tgtgcttggat acgtcttggat acgtcttggat	tgatggatat ttaggggaga gttgtagctt ggggatgtag ctctgaagaa gggtccgcca gcgtaaagca gtgccggtgg aatggcctgt gaggaatgtt atgccttttc tcctgaggta ccaggttcca ttgcccgcac gggaaaactt ctttcagctg acagggcgtc ggaagacccg cctttggacg atgggtact actaggaca actaggaca gaactttatc
2821 2881 2941 3001 3012 3181 3241 3361 3421 3561 3601 3721 3781	tggttataat ttttggtttc tttctatgcg atgcatattc ctgcgcttct taacattgcatt gcattgtaat ttttgatcac tatgccttac cagaatgagc tgatgatacg gccggtgtgt tggagcagag ggggtggggt	gtagtcggta ggaatagtgt aacaatacct tgttggattg caaagatgta acagatactg tgcggtgctt atgctggcta aatgtgttga cagtgtaaca ctaacagaga gtagatgtga ttcggatcca ttcagatgg aaacgcttct ggcaggagtt caattctca cgtggctaat cgtggctaat	tggaagcagt ttatggccaa gtgtagatgc ccacagctgg acctgggcat gatgttat ccgatgagat ccaagtgtac tgaatcatgt tctttgacat tctttgacat gtggagaaga ctgaagatct gtggagaaga ccgaagatg cgtcagaacg cgtcagaacg	aacttttgta taccaaactt ctggggacag cagaaccaag tctgaatgaa tttaattaag gccttatcaa tgtttcccat catgcatgca gaaagtgttg gaacatgcga gagaccggat aactgcgat aactgactaa taaaaatttg gagtcttcag ttatgggatc atgctacttt ccgctaacac ctataaccc ctttgaccca	agatgctgca aatgttaagt atattgcatg gttagtgtac agtcaattgt ggcgaagcaa ggcaatgcca atgctcactt caacgcaaaa ggtgggcgta ttggaaccag atctggaacatg catttggta ggtaggatat tttttctgt cccttatctg tactgtggat agttcttca tgtgcttggat agttcttca tgtgcttggat acgtcttggat acgtcttggat	tgatggatat ttaggggaga gttgtagctt ggggatgtag ctctgaagaa gggtccgcca gcgtaaagca gtgccggtgg aatggcctgt gaggaatgtt atgccttttc tcctgaggta ccaggttcca ttgcccgcac gggaaaactt ctttcagctg acagggcgtc ggaagacccg cctttggacg atgggtact actaggaca actaggaca gaactttatc

						<del></del>
3901	aaaaaaaaat	tccacaatca	atgaataaat	aaacgagctt	geegeegale	LadaalCaay
3961	tgtttttatt	tcatttttcq	cgcacggtat	gccctagacc	accgatctcg	atcattgaga
4001	acacggtgga	ttttttaaa	aatcctatac	addtaggatt	gaatgtttag	atacatgggc
4021	acacggugga	Litticeag	aaccccacag	aggraggace	gaacgcccag	acacacggge
4081	attaggccat	ctttggggtg	gagatagctc	cattgaaggg	attcatgctc	cggggtagtg
4141	ttgtaaatca	cccagtcata	acaaggtcgc	agtgcatggt	gttgcacaat	atcttttaga
4001	agtaggctga		taaggatta	atataaatat	ttacaaacca	attaaactaa
4201	agtaggctga	ttgccacaga	Laageceering	granda	Lucadacty	gregagergg
4261	gaggggtgca	ttcggggtga	aattatgtgc	attttggatt	ggatttttaa	gttggcaata
1221	ttgccgccaa	gatetegtet	tagattcata	ttatgaagga	ccaccaagac	ggtgtatccg
4321	Ligitigueda	gaccicgece	cgggcccacg			***
4381	gtacatttag	gaaatttatc	gtgtagcttg	gatggaaaag	cgtggaaaaa	tttggagata
4441	cccttgtgtc	ctccgagatt	ttccatqcac	tcatccatga	taatagcaat	ggggccgtgg
4501	gcagcagcgc	=======================================	attocataca	teteseset	catacttato	ttcctgagtt
450T	geageagege	gggcaaacac	guccegugg	ccegacacac	tacagooatg	
4561	aaatcatcat	aagccatttt	aatgaatttg	gggcggagag	taccegattg	gggcacgaac
4621	gttccttcgg	accccaaac	atagttcccc	tcacagattt	gcatttccca	agctttcagt
4601	tccgatggtg		asaataaaaa	actatasaas	acaccuttte	tagaacaaaa
4081	ceegatggtg	gaaccacycc	cacciggggg	gccacgaaga		
4741	gtgattagtt	gggatgatag	caagtttctg	agcaattgag	atttgccaca	teeggtgggg
4801	ccataaatga	ttccgattac	aggttgcagg	tggtagttta	gggaacggca	actgccgtct
40.61	tctcgaagca	000030000	ataattaata	atttccctta	catocatatt	ttcccacacc
4861	tetegaagea	agggggccac	Cicquicate	acceccea	- to to the set of	
4921	aaatccatta	ggaggcgctc	tcctcctagt	gatagaagtt	cttgtagtga	ggaaaagttt
4981	ttcagcggtt	ttagaccgtc	agccatgggc	attttggaga	gagtttgctg	caaaagttct
5041	agtctgttcc		astatattat	ataggatata	ratroagrag	acctectect
504I	agtetgttee	acagttcagt	gatgtgtttt	acggcacccc	gatteagtag	accecege
5101	ttcgcgggtt	tggacggctc	ctggagtagg.	gtatgagacg	atgggcgtcc	agcgctgcca
5161	gggttcggtc	cttccagggt	ctcagtgttc	gagtcagggt	tatttccatc	acagtgaagg
2101	gggcccggcc	tt		+~~~~	agtaattata	ataataaaa
5221	ggtgtgcgcc	tgcttgggcg	cttgccaggg	tgegetteag	actuations	Crygraya
5281	acttctgtcg	cttaacaccc	tgtatgtcgg	ccaagtagca	gtttaccatg	agttcgtagt
E2 / 1	tgagcgcctc	aactacataa	cctttaacac	ggagettace	tttggaagtt	ttcttgcata
3341	tgagegeete	ggctgcgtgg	cccccggcgc	9949020400		anttotaga
5401	ccgggcagta	taggcatttc	agcgcataca	gerraggege	aaggaaaatg	gattergygg
5461	agtatgcatc	tacaccacaa	gaggcgcaaa	cagtttcaca	ttccaccagc	caggttaaat
EE 21	ccggttcatt	acactesses	acaacttttc	caccatattt	tttgatgcgt	ttcttacctt
3321	Coggillatt	ggggccaaaa	acaageeeee	teresees		teeeestass
5581	tggtctccat	gagttcgtgt	cctcgttgag	tgacaaacag	getgteegta	teeeegtaga
5641	ctgattttac	aggeetette	tccaqtqqaq	tacctcagtc	ttcttcgtac	aggaactctg
F701	accactctga	tagaaagggg	cacataceaaa	ccaccacaaa	ggaggctatg	taggagggt.
2/07	accactetga	Lacadaggcg	cycyccagg	ccagcacaaa	ggaggeracg	
5761	agcgatcgtt	gtcaaccagg	gggtccacct	tttccaaagt	atgcaaacac	atgteaecct
5821	cttcaacatc	caggaatgtg	attoocttot	aggtgtattt	cacgtgacct	ggggtccccg
5021	ctgggggggt		acceptate	actattacta	actatatta	ggatcgctgt
288T	crgggggggc	acaaaagggg	geggetetet	gerereere	accyccicc	ggategetgt
5941	ccaggaacgt	cagctgttgg	ggtaggtatt	ccctctcgaa	ggcgggcatg	acctctgcac
6001	tcaggttgtc	agtttctaag	aacgaggagg	atttgatatt	gacagtgccg	gttgagatgc
6061	ctttcatgag	attttaataa	atttaataaa	222C2C22t	ttttttt	tcaagtttgg
POPT	ctttcatgag	gerelegice	acceggicag	aaaacacaac	LLCCCCaccy	ccaageeegg
6121	tggcaaatga	tccatacagg	gcgttggata	aaagtttggc	aatggatcgc	acggtttggt
6181	tcttttcctt	atccacacac	tetttggcag	cgatgttgag	ttggacatac	tegegtgeta
6041	ggcacttcca	55555555	atacttatas	attestates	caccattete	acttoccacc
924T	ggcacttcca	EECGGGGGaag	atagitytta	acceatery	cacyattete	accegecace
6301	ctcgattatg	caaggtaatt	aaatccacac	tggtggccac	ctcgcctcga	aggggttcgt
6361	tggtccaaca	gaggetaget	cctttcctag	aacagaaagg	aggaagtggg	tctagcataa
6421	gttcatcggg	Sagetatass	togatogtaa	agattcccgg	aagtaaatcc	ttatcasast .
6421	gricaleggy	ayyytttyta	LCCatggtaa	agatteetegg	hannahan	
6481	agctgatggg	agtggggtca	tctaaggcca	tttgccattc	tcgagctgcc	agrgeaeger
6541	catatgggtt	aaggggactg	ccccagggca	taggataggt	gagtgcagag	gcatacatgc
CC01	cacagatgtc	atagagatag	ataggatect	casadataca	tatatamott	ggataggatg
POOT	Cacagacyce	acayacycay	acgggaccec	caaagacgcc		ggatageatt
6661	gccccctct	gatacttgct	cgcacatagt	catatagtic	acgigatggc	gcraycaacc
6721	ccggacccaa	attaatacaa	ttgggttttt	ctgttctgta	gacaatctgg	cgaaagatgg
6701	cgtgagaatt	acasasta	atagatettt	rassatott	naaatnooca	tgaggtagac
0/07	Cytyayaatt	gyaayayaty	gugggueett	gadadacgcc		
6841	ctacagagtc	tctgacaaag	tgggcataag	attettgaag	cccggccacc	agricageag
6901	tgacaagtac	atctagggca	cagtagtcaa	atatttctta	aatgatgtca	taacctggtt
0001	ggtttttctt	++00000000	tagaaattaa	gaaggtatte	ttcacastcc	ttccagtact
PACT	ggittittett	Licecatage	regeggeega	gaaggcaccc	teegegatee	
7021	cttctagcgg	aaacccgtct	ttgtctgcac	ggtaagatcc	tagcatgtag	aactyattaa
7081	ctgccttgta	agggcagcag	cccttctcta	cgggtagaga	gtatgcttga	gcagcttttc
71 /1	gcagcgaagc		acasacatat	ctctgaccat	dactttdada	aattootatt
1147	ycaycyaagc	gragraagg	gcgaaggcgt	coccyactat	gaccicgaga	
7201	tgaagtccat	gtcgtcacag	getecctgtt	cccagagttg	gaagtctacc	cgtttcttgt
7261	aggcggggtt	gggcaaagcg	aaagtaacat	cgttgaagag	aatcttacco	gctctgggca
7221	taaaattgcg	200	assunctata	atacttcccc	tegattotto	atcacctoon
1261	LadadLLycg	agryarycyy	aaayyccycy	gracectic	togattytty	
7381	cagctaggac	gatctcgtcg	aaaccgttga	tgttgtgtcc	tacgatgtat	aattctatga
7441	aacgcggcgt	geetttgaeg	tgaggtagct	tattgagctc	atcaaaggtt	aggtctataa
7501	ggtcagataa		toracacco	attecteese	atagaattt	acetateace
7561	atgatgacca	aagatccacc	gccagtgctg	tttgtaactg	gtcccgatac	tgacgaaaat
7621	gctggccaat	taccattttt	tetagaataa	cacagtagaa	gattctagag	tcttgttacc
7601	atcgatccca	atttaattta	etaacteast	catagagagat	attaaccaca	cactettata
100T	accyarccca	CLLCagttta	acygerayat	cycygyccat	guugauga	
7741	ctgagagttt	catgaccagc	atgaaaggaa	ctagttgttt	gccaaaggac	cccatccagg
, ,						

FIG. 28A-2

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7801	tgtaagtttc	cacatcotao	gtcaggaaga	atctttctat	gcgaggatga	gageegateg
7001	ggaagaactg		gaaaattaa	aggattaget	attastatas	togaantaga
\ Q O T	ggaagaaccg	gattteetge	caccagergg	aggactggcc	geegaegega	cggaag caga
7921	agtttctgcg	acacaccasa	cattcgtgtt	tgtgcttgta	cagacggccg	cagtagtcgc
7001	agcgttgcac	acattatata	tcatastas	actataceta	actteectta	acqaqaaatt
IART	ageguigeae	gggttgtate	ccgrgaacga	geegeaceeg	geeeeee	
8041	tcagtgggaa	geegaggeet	ggcgattgta	tetegtgete	ttctatattc	gctgtatcgg
0101	cctgttcatc	ttetetttee	ataataatca	tactaacaaa	cccccacaaa	aggcaagtcc
9101	Congrecate	cccgcccg	araaraarra	cgccgacgag		esetetes:
8161	agacctcggc	gcgggagggg	cggagctgaa	ggaccagagc	gegeaggeeg	gagetgteea
8221	gagtcctgag	acoctocooa	ctcaggttag	taggtaggga	cagaagatta	acttgcatga
0001	5490000949		acettanest	gataattaat	ttecacacat	teatttataa
828T	tcttttccag	ggegrgeggg	aggillagal	ggtacttgat	CCCacagge	cogcogcag
8341	agatgtcaat	ggcttgcagg	gttccgtgtc	ctttgggcgc	cactaccgta	cctttgttt
9401	ttcttttgat	caataataac	tetettaett	cttgcatgct	cagaagcgat	gacggggacg
0401	ccccccgac	caaraaraac	cccccgccc			senseresse
8461	cgcgccgggc	ggaagcggtt	gttccggacc	cggaggcarg	gctggtagtg	geacgregge
8521	gccgcgcacg	ggcaggttct	ggtactgcgc	tctgagaaga	cttacataca	ccaccacgcg
0501	tcgattgacg	tottotatat	gacatatata	aataaaact	accaacccca	tgagettgaa
929T	tegattgatg	Colligiates	gacycccccg	ggtgaaaget		
8641	cctgaaagag	agttcaacag	aatcaatttc	ggtatcgtta	acggcagctt	geeceageae
8701	ttcttgtacg	tcaccagagt	totcctoota	ggcgatctcc	gccatgaact	gctcgatttc
0761	ttcctcctga	agatatagaga	ascecetet	ctccaccata	accacaaaat	cattggagat
9/0T	Liceteetga	agateteege	gaccegeree	cccgacggcg	geegegagge	
8821	acggcccatg	agttgggaga	atgcagtcat	geeegeeteg	ttccagacgc	ggctgtaaac
8881	cacggccccc	teggagtete	ttgcgcgcat	caccacctga	gcgaggttaa	gctccacgtg
0001	tctggtgaag	aggaatagt	tacatacaca	ctassasaa	tagttgagtg	tootoocaat
0941	terggraag	accycatage	Lycalaggeg	ccgaaaaagg		terestagg
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10561 10621 10681 10741 10801 10861 10921 10981 11041 11161 11221 11281 11341 11401	tactcgagcc tacaaaaatc agtcctattt acaacagccc tgcgctgtg actggcacgt ttctcgcgag ggaggagatg aagacgagtg cagggcacac gcgtaatttc caccttggt caaacctctg cagagaggcg cattctacag catcaattac	tggaggaacg ggccggagcc caggatacgg ttttttttg ccctcgcagc agcggtgcgg ctaggtgcg gcgtatgtgc cgagcttccc ttgcgggacg gtggctgcag caaaagtctt ttgatgcatt accgcacagc ctgctcaacag tcgttttga agtatcatag tcggttttga atagacaagg	tgaacgggtt gcggctaacg aatcgagtcg ccgctcagat agcagcacc gacagccgc cctcgccga gctttaacgc aggatttcga ccaaccttgt ttaataatca tgtgggattt tgtttcggt tcacgaacc tgcaggaggg gcttgggaaa aggtgaagat	gggtcgcggt tggtattggc ttttgctggt gcatcccgtg acaaaaggct ctatgatctg gcggcatccg cctatttaga gggtcgtgag agttgatgaa atcggcttac tgtgcgaacc gatggaagct ggtgcaacac ggtgcaacac ggtgcaacac ggtgcaacac ggtgcaggag gtattacgct agatgggtt	gaaagcgttc gtaccccggt actcccgtct tgccgaatgg ctgcgacaga gtccctgcaa gacttggaag cgagttcaac gacagaagcg ctgcgtcacg gtgacaggga gaacagacag ctcattgccc atcattcaga agcagagacat ggtgtgata ctggcgagac tggtgtgata ctgatgatat ctggccgaga ctcattgccc	agcgactcga tcgagacttg cgaccagcc cagggaagtg tgcgtccca agggcgaagg tgaaaaaaga gcgaggagcc gtttggacag tcagtcctgc taaaggaaga gcgaagaagt acctactag atgaggettt atcttatcaa aggtggctgc tgacagactcc tgacgctgaa
10561 10621 10681 10741 10801 10861 10921 10981 11041 11161 11221 11281 11341 11401	tactcgagcc tacaaaaatc agtcctattt acaacagccc tgcgctgtg actggcacgt ttctcgcgag ggaggagatg aagacgagtg cagggcacac gcgtaatttc caccttggt caaacctctg cagagaggcg cattctacag catcaattac	tggaggaacg ggccggagcc caggatacgg ttttttttg ccctcgcagc agcggtgcgg ctaggtgcg gcgtatgtgc cgagcttccc ttgcgggacg gtggctgcag caaaagtctt ttgatgcatt accgcacagc ctgctcaacag tcgttttga agtatcatag tcggttttga atagacaagg	tgaacgggtt gcggctaacg aatcgagtcg ccgctcagat agcagcacc gacagccgc cctcgccga gctttaacgc aggatttcga ccaaccttgt ttaataatca tgtgggattt tgtttcggt tcacgaacc tgcaggaggg gcttgggaaa aggtgaagat	gggtcgcggt tggtattggc ttttgctggt gcatcccgtg acaaaaggct ctatgatctg gcggcatccg cctatttaga gggtcgtgag agttgatgaa atcggcttac tgtgcgaacc gatggaagct ggtgcaacac ggtgcaacac ggtgcaacac ggtgcaacac ggtgcaggag gtattacgct agatgggtt	gaaagcgttc gtaccccggt actcccgtct tgccgaatgg ctgcgacaga gtccctgcaa gacttggaag cgagttcaac gacagaagcg ctgcgtcacg gtgacaggga gaacagacag ctcattgccc atcattcaga agcagagacat ggtgtgata ctggcgagac tggtgtgata ctgatgatat ctggccgaga ctcattgccc	agcgactcga tcgagacttg cgaccagcc cagggaagtg tgcgtccca agggcgaagg tgaaaaaaga gcgaggagcc gtttggacag tcagtcctgc taaaggaaga gcgaagaagt acctactag atgaggettt atcttatcaa aggtggctgc tgacagactcc tgacgctgaa
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10561 10621 10681 10741 10861 10981 10981 11041 11161 11221 11281 11341 11401 11461 11521	tactcgagcc tacaaaaatc agtcctattt acaacagccc tgccgctgtg actggcacgt ttctcgcgag ggaggagatg cagggcacac gcgtaatttc caccettggt caaacctctg cagagaggcg cattctacag catcattac atacgttgccagg	tggaggaacg ggccggagcc caggatacgg ttttttttg ccctcgcagc agcggtgcgc gcgatatgtgc ctgggcttccc ttgcgggacg gtggctgcag caaaagtctt ttgatgcatt accgcacagc ctgctcaaca agtatcatag tcggtttga atagacaagg	tgaacgggtt gcggctaacg aatcgagtcg ccgctcagat agcagcacc gacagccgc cttcgccga ccaacagaa gctttaacgc aggatttcga ccaaccttgt ttaataatca tgtgggattt tgttctggt tcaccgaacc tgcaggacg gcttgggaaa aggtgaagat acttggggt	gggtcgcggt tggtattggc ttgttgctggt gcatcccgtg acaaaaggct ctatgatcg gcggcatccg cctatttaga gggtcgtgag agttgatgaa atcggcttac tgtgcgaacc gatggaagct ggtgcaacac cgaggggaga gagcctgggc gtattacgct agatgggtc	gaaagcgttc gtaccccggt actcccgtc tgccgaatgg ctgcgacaga gtccctgcaa gacttggaag cgagttcaac gacagaagcg gtgacagga gacagacag gacagacag ctcattcaga agcagagaca tggttgtatg ctggccgaga cgcaagatct tacatgccc gacagacatgcc gacagacatg	agcgactcga tcgagacttg cgacccagcc cagggaagtg tgcgtcccca ctactgcaac agggcgaagg tgaaaaaaga gcgaggagcc gtttggacag tcagtcctgc tacagtcctgc tacagagaaga accctactag atgaggcttt atcttatcaa aggtggctgc acaagactcc tacgcgctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa atgaggctgaa
10561 10621 10681 10741 10861 10981 10981 11041 11161 11221 11281 11341 11401 11461 11521	tactcgagcc tacaaaaatc agtcctattt acaacagccc tgccgctgtg actggcacgt ttctcgcgag ggaggagatg cagggcacac gcgtaatttc caccettggt caaacctctg cagagaggcg cattctacag catcattac atacgttgccagg	tggaggaacg ggccggagcc caggatacgg ttttttttg ccctcgcagc agcggtgcgc gcgatatgtgc ctgggcttccc ttgcgggacg gtggctgcag caaaagtctt ttgatgcatt accgcacagc ctgctcaaca agtatcatag tcggtttga atagacaagg	tgaacgggtt gcggctaacg aatcgagtcg ccgctcagat agcagcacc gacagccgc cttcgccga ccaacagaa gctttaacgc aggatttcga ccaaccttgt ttaataatca tgtgggattt tgttctggt tcaccgaacc tgcaggacg gcttgggaaa aggtgaagat acttggggt	gggtcgcggt tggtattggc ttgttgctggt gcatcccgtg acaaaaggct ctatgatcg gcggcatccg cctatttaga gggtcgtgag agttgatgaa atcggcttac tgtgcgaacc gatggaagct ggtgcaacac cgaggggaga gagcctgggc gtattacgct agatgggtc	gaaagcgttc gtaccccggt actcccgtc tgccgaatgg ctgcgacaga gtccctgcaa gacttggaag cgagttcaac gacagaagcg gtgacagga gacagacag gacagacag ctcattcaga agcagagaca tggttgtatg ctggccgaga cgcaagatct tacatgccc gacagacatgcc gacagacatg	agcgactcga tcgagacttg cgacccagcc cagggaagtg tgcgtccca cagggcgaagg tgaaaaaaga gcgaggagcc gtttggacag tcagtcctgc taaaggaaga gcgaagaagt accctactag atgaggctta atctattata aggtggctgc tacagctcag acagactcc tgacgctgaa atcgcgcggt

11701	gcagcctagt	cgcagggctc	tgaacgccgc	gacggcagga	tgtgagcttc	cttacataga
11761	agagggggat	gaaggcgagg	aggaagaggg	cgagtacttg	gaagactgat	ggcacaaccc
11821	atattttta	ctagatggaa	cagcaagcac	cggatcccgc	aatgcgggcg	gcgctgcaga
11881	accaaccate	coocattaac	tecteggacg	attggaccca	ggccatgcaa	cgtatcatgg
11941	cgttgacgac	tcgcaacccc	gaagccttta	gacagcaacc	ccaggccaac	cgtctatcgg
12001	ccatcatgga	agctgtagtg	ccttcccgct	ctaatcccac	tcatgagaag	gtcctggcca
12061	tcgtgaacgc	gttggtggag	aacaaagcta	ttcgtccaga	tgaggccgga	ctggtataca
12121	accetetett	agaacgcgtg	gctcgctaca	acagtagcaa	tgtgcaaacc	aatttggacc
12181	gtatgataac	agatgtacgc	gaagccgtgt	ctcagcgcga	aaggttccag	cgcgatgcca
12241	acctgggttc	gctggtggcg	ttaaatgctt	tcttgagtac	tcagcctgct	aatgtgccgc
12301	gtggtcaaca	ggattatact	aactttttaa	gtgctttgag	actgatggta	tcagaagtac
12361	ctcagagcga	agtatatcag	tccggtcctg	attacttctt	tcagactagc	agacagggct
12421	tgcagacggt	aaatctgagc	caagctttta	aaaaccttaa	aggtttgtgg	ggagtgcaty
12481	ccccggtagg	agaaagagca	accgtgtcta	gettgttaae	teegaaetee	cgcctattat
12541	tactgttggt	agctcctttc	accgacagcg	gtagcatcga	cegtaattee	caccigggic
12601	acctactaaa	cctgtatcgc	gaagccatag	ggcaaagtca	ggtggacgag	ttggacctacc
12661	aagaaattac	ccaagtcagt	cgcgctttgg	gacaggaaga	tactosstat	actettacta
12721	ctctgaactt	cttgcttacc	aatcggtctc	aaaagacccc	gggattatt	ctactacta
12781	cggaggagga	gaggatcctt	agatatgtgc	tanagagagaga	asstatorar	cccaccatat
12841	agggggcaac	tccgactgca ccgacctttc	gcaccygaca	tratageges	cttccacaca	actaccacta
12301	atgccagtaa	ttatttcacc	actaacaaac	taaaccccc	ctagetacea	ccacctaatt
12301	tgaactetga	cgaatatgac	atroccasco	ctaatcacca	atttctgtgg	gacgacgtgg
13021	teracacggg	ttttcacct	ctttctcatc	atcacacata	gaaaaaggaa	ggcggcgata
13141	acagegatyt	ttctgcatcg	ctatccaaaa	trattagtag	taccocooct	gagcccgagt
13301	gaatycattc	ttttcctagt	ctaccctttt	ctctacacag	totacotacc	agcgaagtgg
13201	gtagataag	tcgcccgagt	ttaatgggcg	aagaggagta	cctaaacgat	tecttactca
13331	gragaaraag	agaaaaaaat	ttcccaaaca	atggaataga	aagtttggtg	gataaaatga
13321	gaccygcaag	gacttatgct	caggatcaca	gagacgagcc	taggatcata	gggactacaa
13441	gtagatggaa	ccgtagacgc	cagcgccatg	acagacagag	agatettata	tgggacgatg
13501	aggattcggc	cgatgatagc	agcgtattgg	acttgggtgg	gagaggaagg	ggcaacccgt
13561	ttoctcattt	acaccctcac	ttgggtggta	tgttgtaaaa	aaaaataaaa	aagaaaaaac
13621	tcaccaagge	catggcgacg	agcgtacgtt	cgttcttctt	tattatctgt	gtctagtata
13681	atgaggggag	tcatactaga	cggagcggtg	gtgtatccgg	agggtcctcc	tccttcgtac
13741	gagagcgtga	tocaocaoca	gcaggcgacg	gcggtgatgc	aatccccact	ggaggctccc
13801	tttatacctc	cocoatacct	ggcacctacg	gagggcagaa	acagcattcg	ttactcggaa
13861	ctggcacctc	agtacgatac	caccaggttg	tatctggtgg	acaacaagtc	ggcggacatt
13921	gettetetga	actatcagaa	tgaccacage	aacttcttga	ccacggtggt	gcaaaacaat
13981	gactttaccc	ctacggaagc	cagcacccag	accattaact	ttgatgaacg	atcgcggtgg
14041	ggcggtcagc	taaaaaccat	catgcatact	aacatgccca	acgtgaacga	gtatatgttt
14101	agtaacaagt	tcaaagcgcg	tgtgatggtg	tccagaaaac	ctcctgaggg	tgttagagta
14161	gacgataatt	atgatcataa	gcaagatatt	ctaaaatacg	agtggttcga	gtttactttg
14221	ccagaaggca	acttttcggt	cactatgact	ategaettga	cgaacaatge	tattaaatta
14281	aattacttga	aagtgggcag	acagaatgga	gtgttggaaa	gracarray	gctaagtt
14341	gacactagga	acttcaagtt	gggatgggat	ccagaaacta	atacatacaa	agtggagtt
14401	tacacctatg	aggccttcca gtctgagcaa	anttattaga	attacasaca	aacacccatt	ccaagagggt
14401	accgaaagcc	tgtatgagga	tttagaagga	grasatattc	cagccctttt	ggatgtagat
14221	cottatgacci	acagcaagaa	aratraaaaa	gccaaaatag	aagctgctgc	agaagctaaa
14501	gcccacgaga	ttgccaacga	tccaataaaa	gtggctaacg	ctagtgaaat	caggggagac
14701	artttacca	caacatccgt	tecgaetaaa	gaatcattat	togatgatgt	otctcaaaac
14761	ataganttaa	aactcactat	taagcctgtg	gaaaaagatg	gcaaaaacag	aagttacaat
14821	atattagaaa	ataaaatcaa	cacggcctat	cacaattagt	acctttcgta	caattatggc
14881	gegeeggaag	aaggagtgcg	ttcctggaca	ttgctcacca	cctcagatgt	cacctgcgga
14941	acadaacaaa	tctactggtc	gcttccagac	atgatgcagg	atcctgtcac	tttccgctcc
15001	actagacaag	tcagtaacta	ccctataata	ggtgcagagc	ttatgcccgt	cttttcaaag
15061	agettetaca	acqaacaaqc	tototactcc	cagcagetee	gccagtccac	ctcgcttacg
15121	cacqtcttca	accoctttcc	tgagaaccag	attttaatcc	gteegeegge	gcccacaatt
15181	accaccotca	gtgaaaacgt	tcctqctctc	acagatcacg	ggaccctgcc	gttgcgcagc
15241	agtatccggg	gagtccaacg	tataaccatt	actgacgcca	gacgccgcac	ctgtccctac
15301	atatacaaga	cactgggcat	agtcgcaccg	cgcgtccttt	caagccgcac	tttctaaaaa
15361	аааааааааа	atotecotte	ttatctcgcc	cagtaataac	accggttggg	gtctgcgcgc
15421	teccageaag	atgtacggag	gcgcacgcaa	acgttctacc	caacatcccg	tgcgtgttcg
15481	coggocatttt	cacactccat	aaaataccct	caagggccgc	actcgcgttc	gaaccaccgt
15541	cgatgatgta	atcgatcagg	tggttgccga	cgcccgtaat	tatactccta	ctgcgcctac
						•

FIG. 28A-4

15601	atctactgtg	gacgcagtta	ttgacagtgt	agtggctgac	gctcgcaact	atgctcgacg
15661	taanannna	cgaaggcgca	ttaccagaca	traccdaget	accactocca	tacaaacaac
12001	caayayccyy	cgaaggegea	-t		coccepta	2222222
15721	aagagctctg	ctacgaagag	ctagacgcgt	ggggcgaaga	gecatgetta	aaacaaccaa
15781	acgtgcagct	tcgggcgcca	gcgccggcag	gtcccgcagg	caagcagccg	ctgtcgcagc
15841	ggggggtatt	gccgacatgg	cccaatcgcg	aagaggcaat	gtatactggg	tgcgtgacgc
12041	ggcgaccacc	9009404099	cochacogog	angtoccot	cacacttaca	agatactgag
12201	tgccaccggt	caacgtgtac	eegtgegeae	Ceguecece	cycactiaga	agacaccgag
15961	cagtctccga	tgttgtgtcc	cagcggcgag	gatgtccaag	cgcaaataca	aggaagaaac
16021	actacagatt	atcgcacctg	aagtctacgg	ccaaccqttq	aaggatgaaa	aaaaaccccg
1 0001		cgggtaaaaa	244242222	20220002	gatggggatg	atgractage
TOORT	caaaatcaag	Cyyyraaaaa	ayyacaaaaa	agaagaggaa	bacagegaeg	
16141	ggagtttgtg	cgcgagtttg	ccccacggcg	acgcgtgcaa	rggcgrgggc	gcaaaguucg
16201	acatototto	agacctggaa	cttcggtggt	ctttacaccc	ggcgagcgtt	caagcgctac
16261	ttttaagggt	tcctatgatg	aggtgtacgg	ggatgatgat	attettgage	aggcagctga
10201	LLLLaagtyL		aggegeaegg	tostosacs	2010003230	atraaarart
16321	ccgattaggc	gagtttgctt	arggcaageg	caytayaata	aaccccaagg	atyaaacage
16381	gtccataccc	ttggatcatg	gaaatcccac	ccctagtctt	aaaccggtca	ctttgcagca
16441	agtgttaccc	gtaactccgc	gaacaggtgt	taaacqcqaa	ggtgaagatt	tgtatcccac
16501	tatacaacta	atggtgccca	aacuccanaa	attagaggac	gttttggaga	aagtaaaagt
10201	Lacycaacty	atggtgttta	bbasse	gccggaggac	3000033-34	cacctaatet
16561	ggatccagat	attcaacctg	aggttaaagt	gagacccatt	aagcaggcag	cgcctggtct
16621	gggagtacaa	actgtagaca	ttaaaattcc	cactgaaagt	atggaagtgc	aaactgaacc
16681	cocaaaocct	actgccacct	ccactgaagt	gcaaacqqac	ccatggatgc	ccatgcctat
16741	tagaaataaa	gccgtcggtc	ccactccaac	atcccracca	aagtacggtc	cagcaagtet
10/41	Lacaactgac	gccgccggcc	t	Lattattast	aagtatggtt	2000200000
16801	gttgatgccc	aactatgtcg	tacacccatc	tattatteet	actectggtt	accyaggeac
16861	tcgctactat	cgcagccgaa	acagtacttc	ccgccgtcgc	cgcaagacac	ctgcaaatcg
16921	cagtegtege	cgtagacgca	caagcaaacc	gattcccggc	gccctggtgc	ggcaagtgta
1 6001	aagaaataat	agtgcggaac	ctttcacact	accacataca	cottaccatc	ctagtatcat
10301	ccycaacygc	agtgtggaat			cgttactact	agaattagaa
17041	cacttaatca	atgttgccgc	tgcctccttg	cagatatggc	ceteaerige	cyccutcycy
17101	ttcccatcac	tggttaccga	ggaagaaact	cgcgccgtag	aagagggatg	ttggggcgcg
17161	gaatgcgacg	ctacaggcga	coocotocta	tecgcaagea	attocoogot	ggttttttgc
17701	gaacgcgacg	tccaattatc	actactacas	ttaacacaat	accardeata	acttecataa
1/221	Cagcertaar	LCCaartacc	getgetgega	coggogodac	accaggeata	224222322
17281	cggttcaggc	ctcgcaacga	cattgacatt	ggaaaaaaaa	aaaacgtata	aataaaaat
17341	acaatqqact	ctgacactcc	tggtactgtg	actatgtttt	cttagagatg	gaagacatca
17401	atttttcatc	cttggctccg	cgacacggca	cgaagccgta	catgggcacc	tggagcgaca
17461	***************	ccaactgaac	Tagggggggggg	traattagag	cantateton	agegggetta'
1/461	ceggeacgag	ccaactyaac	gggggcgccc	ccaaccygag	theresees	agegggeeea
17521	aaaattttgg	ctcaaccata	aaaacatacg	ggaacaaagc	ttggaacagc	agtacaggac
17581	aggcgcttag	aaataaactt	aaagaccaga	acttccaaca	aaaagtagtc	gatgggatag
17641	cttcccctat	caatggagtg	gtagatttgg	ctaaccagge	totocagaaa	aagataaaca
17701	ctcccggcac	cccgccgcca	2002000000	atassatass	autonagnaa	gaaatteete
T / / OT	gtegttigga	ecegeegeea	gcaaccccay	gryadaryca	ageggaggaa	shaataaaa
17761	cgccagaaaa	acgaggcgac	aagcgtccgc	gtcccgattt	ggaagagacg	ciggigacge
17821	gcgtagatga	accgccttct	tatgaggaag	caacgaagct	tggaatgccc	accactagac
17881	chatanecee	tatggccacc	ggggtgatga	aaccttctca	gttgcatcga	cccgtcacct
17001	terestitees	acatactact	ggggggacga	ctatacccac	ttctaacct	atcactaccc
1/941	tggatttgcc	ccctcctcct	gergeracing	ClyLactege	tectaageee	gaagaagaaa
18001	cgaaaccagt	cgccgtagcc	aggtcacgtc	ccgggggcgc	tcctcgtcca	aatgcacact
18061	ggcaaaatac	tctgaacagc	atcgtgggtc	taggcgtgca	aagtgtaaaa	cgccgtcgct
19121	acttttaatt	aaatatggag	taggggttaa	cttgcctatc	tototatato	totcattaca
10121	gccccaacc		22222222	coagtactac	atageaacta	agttactttc
TRIRI	cgccgtcaca	gcatcagagg	aaaaaayyaa	gaggicgige	gucgacgurg	agucacccc
18241	aagatggcca	ccccatcgat	gctgccccaa	tgggcataca	tgcacatcgc	cggacaggat
18301	gcttcggagt	acctgagtcc	gggtctggtg	cagttcgccc	gcgccacaga	cacctacttc
18361	aatctoooaa	ataagtttag	aaatcctacc	gtagcgccga	cccacgatgt	gaccaccgat
10301	aattegggaa	acatestat	gagattagta	googttgcc	aaaaaaaaa	tacatactct
18421	egtageeage	ggctcatgtt	gegertegtg	CCCyccyacc	gggaggacaa	
18481	tacaaagtgc	ggtacaccct	ggccgtgggc	gacaacagag	tgctggatat	ggccagcacg
18541	ttctttgaca	ttaggggcgt	gttggacaga	ggtcccagtt	ttaaacccta	ttctggtacg
18601	acttacaact	ccctggctcc	taaaggcgct	ccaaatgcat	ctcagtggtt	ggataaggga
10001	-tt	ctggcctagt	22222222	aatactgatg	ataaaaaaa	2000233333
TROOT	gttacaagca	etggeetagt	ggacgacggc	aatactgatg	acggggaaga	agccauaaaa
18721	gcaacataca	cttttggtaa	tgctccagta	aaagccgagg	ctgaaatcac	aaaagacgga
18781	ttaccaataa	gcttggaagt	ttcaactgaa	ggtcctaaac	caatctatgc	tgataagctt
10041	tatrarrra	aacctcaagt	addadacdaa	acttogacto	acctagacon	aaaaacccaa
1000T	-accaycoay		+0005-	2012332103	aaccctccts	constattt
18301	gagtatggag	ggagggttct	Ladacctgaa	actadaatya	adoctigota	- wa
18961	gctaaaccta	ctaatattaa	aggaggtcag	gcaaaggtaa	aaccaaaaga	agacgatggc
19021	actaacaaca	tcgagtatga	cattgacatg	aacttctttg	acttaagatc	acaaagatca
10001	reactrees.	ctaaaattgt	aatotatora	gaaaatgtoo	acctggaatg	tccagatact
19001	yaacccaaac	occasion to	24555	2444445		tanacanasa
19141	catgttgtgt	acaaacctgg	agtttcagat	gctagetteg	ayaccaacct	Lygacaacag
19201	tctatgccca	acagacccaa	ctacattggc	ttcagagata	. acttcatcgg	acttatgtac
19261	tataacagta	ctggcaacat	qqqqqtactq	gctggccaag	r cgtctcagtt	gaatgcagtg
10321	attasattas	addacadaaa	cacacaacto	tettacease	tettaettaa	ctctctgggc
7336T	gulyactige	assacette-	gatetees - t	and a contract of the	acanttates	tootostots
TA381	gacagaacca	galacticag	cargraggaat	cayyorycyy	acayttatya	tcctgatgta
19441	cgtgttattg	aaaatcatgg	tgtggaagat	gaacttccca	actactgttt	teegttggat
	-					

19501	gatategate	cgcgaacaga	tagttagaag	gagattaagc	caaatggaga	ccaatctact
TADOT	tggacaaatg	Lagacccaac	tggcagcagt	gaactigeta	agggaaatcc	accigccatg
19621	gaaattaacc	ttcaagccaa	tctatggcga	agtttccttt	attccaatgt	ggctctatat
10601	ataccacat	cetacanata	and a garage and	aatataaata	ttccagaaaa	022222000
TAGGT	Cleccayact	Cycacaaca	Caccecgice	aatyttattt	LLCLayaaaa	Caaaaacacc
19741	tacqactaca	taaacaaaca	ggtggtgccg	ccatctctag	tagacaccta	tgtgaacatt
10001	aataaaaat	agtatataga	taccatacac	aatotcaaco	cattcaacca	ccaccotaac
19861	gctggcttgc	gttaccgatc	catgcttctg	ggtaacggac	gttatgtgcc	tttccacata
19921	caagtgcctc	assasttett	cactattess	aacctoctoc	ttctcccagg	ctcctacact
10001	caagegeeee	aaaaaccccc	cgccgccaca	auccegeege	cccccagg	
19981	tatgagtgga	actttaggaa	ggatgtaaac	atggttctac	agagttccct	cggtaacgac
					acctctatgc	
					tgcggaatga	
20161	cagtcattca	acgactacct	atctgcagct	aacatgctct	accccattcc	toccaatoca
					ctttcagagg	
20281	accagactga	aaaccaaaga	aactccctct	ttggggtctg	gatttgaccc	ctacttcgtc
					tgaaccacac	
20401	gtttccatca	tgtttgactc	ttcagtgagc	tggcctggaa	atgacaggtt	actateteet
20461	aacqaatttq	aaataaagcg	cactotogat	ggcgaaggct	acaacgtagc	ccaatgcaac
					acaacatcgg	
20581	ttctacattc	cagaaggata	caaagatcgc	atgtattcat	ttttcagaaa	cttccagccc
					tcaaggccgt	
20701	taccaacaca	acaactctgg	ctttgtgggt	tacatggctc	cgaccatgcg	tcaaggtcaa
20761	ccctatccca	ctaactatcc	ctatccactc	attogaacaa	ctgccgtaaa	tagtgttacg
20821	cagaaaaagt	tcttgtgtga	cagaaccatg	tggcgcatac	cgttctcaag	caacttcatg
20881	tctatgggag	cccttacaga	cttgggacag	aacatgctct	atgccaactc	ageteatget
					ccctgcttta	
21001	gaagttttcg	acataatcaa	agtgcatcag	ccacaccgcg	gcatcatcga	ggcagtctac
					aagcttcttg	
21001	Cigcylacac	Carrereage	Cygraacycr	accacycaag	aagcttcttg	CLLCLigcaa
21121	acagcagctg	caaccatggc	ctgcggatcc	caaaacggct	ccagcgagca	agagctcaga
21181	accattatec	aagacctggg	ttgcggacca	tattttttgg	gaacctttga	taagcgcttc
					taaatacggc	
21301	acggggggag	agcactggtt	aactttcaat	togaacccac	gttctaacac	ctgctacctt
21261	tttaataatt	ttaaattata	ggatgatggt	atassaaas	tttaccagtt	tasstatasa
21421	ggtctcctgc	gccgcagcgc	tcttgctacc	aaggaccggt	gtattacgct	ggaaaaatct
21481	acceanacen	tacaaaaccc	ccattctacc	acctacaaac	ttttctgctg	catottcctt
21541				5000505500		
21241	catgeetttg	tgeactggee	tgacegteee	acggacggaa	accccaccat	gaaattytta
21501	actggagtgc	caaacaacat	gcttcattct	cctaaagtcc	agcccaccct	gtgtgacaat
					attttcgctc	
21/21	cacatcgaaa	gggccactge	gttcgaccgt	arggargrge	aataatgatt	Catgladaca
21781	acgtgttcaa	taaacagcac	tttattttt	acatgtatcg	aggctctgga	ttacttattt
21841	atttacaact	castagatt	ctgacgagaa	tragaatgar	ccgcaggcag	tgatacgttg
21021	acccacage					
7130T	cggaactgat	actiggging	ccacttgaat	tegggaatea	ccaacttggg	aaccygtata
21961	tcqqqcaqqa	tgtcactcca	cagctttctg	gtcagctgca	aagctcccag	caggtcagga
22021	accassatct	traaatrara	attaggagga	atactetasa	cgcgagagtt	acagtacacc
22021	geegaaaeee		actaggacca	gogocoogag		509904040
					cgcttgccag	
22141	tctqcaatca	tgcccacatc	cagatettea	gcattggcaa	tgctgaacgg	ggtcatcttg
					ggttacaatc	
22261	gggatcagta	tcatcttggc	ctgatcctgt	ctgattectg	gatacacggc	tctcatgaaa
22321	gcatcatatt	acttaaaaac	ctactagact	ttactaccct	cggtataaaa	catcccccaq
22201	sasstastas	anagtest	agatagaag	carrantant	tcacacacca	acaaaataa
					tcacacagca	
22441	ttattaacta	tttgcaccac	acttctgccc	cagcggtttt	gggtgatttt	ggttcgctcg
					catccatctc	
					tgccctcata	
22621	ccatgaggcc	acaacgcaca	occtotacat	tcccaattat	ggtgggcgat	ctgagaaaaa
					tcagtgtctt	
					ggtgacagat	
22801	tattcatact	gctcaggcat	tagtttaaaa	gaggttctaa	gttcgttatc	cagectotae
					aagcagacac	
22921	ctaatcggat	tcttaacagt	gcaggcagca	gctcctttag	ccagagggtc	atctttggcg
					gcacgggcgg	
22201			5000000000	tabbattar-	2222-33	
					tgtcttgact	
23101		atttaatett	ccttaacttc	ttttcaaaa	gtatcggagg	aggaggactg
	atogggacar					
			THE THE THE	~~~~++*~~~	FC2CC2CC2~	
53101	tcgctccgtt	ccggagacag	ggaggattgt	gacgtttcgc	tcaccattac	caactgactg
23221	tcgctccgtt tcggtagaag	ccggagacag aacctgaccc	cacacggcga	caggtgtttc	tcttcggggg	cagaggtgga
23221	tcgctccgtt tcggtagaag	ccggagacag aacctgaccc	cacacggcga	caggtgtttc	tcttcggggg	cagaggtgga
23221 23281	tcgctccgtt tcggtagaag ggcgattgcg	ccggagacag aacctgaccc aagggctgcg	cacacggcga gtccgacctg	caggtgtttc gaaggcggat	tcttcggggg gactggcaga	cagaggtgga
23221 23281	tcgctccgtt tcggtagaag ggcgattgcg	ccggagacag aacctgaccc aagggctgcg	cacacggcga gtccgacctg	caggtgtttc gaaggcggat	tcttcggggg	cagaggtgga

FIG. 28A-6

22401			•			
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00101	gogooccoo				22222222	ttaaggattg
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23521	caccocccao	tcctgccacc	acctctaccc	tagaagataa	ggaggtcgac	gcatctcatg
22501		********	aaaaatata	agggagat	cgaacaagac	ccaaactata
7329T	acatgcagaa	caaaaaayyy	aaayayttty	agccagacac	cgaacaagac	ccaaaccaca
23641	tgacaccggt	ggaacacgag	gaagagttga	aacgctttct	agagagagag	gatgaaaact
22701	~~~~	acaaacaaat	aactatcacc	aagatgetgg	aaatagggat	cagaacaccg
23/01	gcccaaaaca	gcaageggae	aactactact	aagacgccgg	aaaaagggaa	
23761	actacctcat	agggcttgac	ggggaagacg	cgctccttaa	acatctagca	agacagtcac
23821	tcatactcaa	ggatgcatta	ttagacagaa	ctgaagtgcc	catcagtgtc	gaagagetea
23021	ccacagecaa	ggacgcacca				500000000
23881	gccgcgccta	cgagcttaac	ctattttcac	ctegtactee	ccccaaacgt	Cayccaaacg
23941	gcacctgcga	gccaaatcct	cocttaaact	tttatccagc	ttttgctgtg	ccagaagtac
24001	**********	tesestatt	tttaaaaata	aaaaaattcc	agtctcctgc	cacactaatc
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24061	qcacccqcqc	cgatgcccta	ctcaatctgg	gacctggttc	acgcttacct	gatatagctt
2/121	cettagaaga	aattccaaaa	atcttccacc	atctagacaa	taatgagact	coooccocaa
24121	ccccggaaga	ggttccaaag	acceccgagg	geoegggeaa		eterestter.
24181	atgctctgca	aaagggagaa	aatggcatgg	atgagcatca	cagcgttctg	grggaarrgg
24241	aaggcgataa	toccagacte	gcagtactca	agcgaagcgt	cgaggtcaca	cactttgcat
24201	aaggegatat	anagatagaa	gatasagtas	tascaaccat	catggaccag	ttactcatta
,243UI	acceegetgt	Caaccigece	CCLadaycca	rgacggccgc	cacggaccag	CCCCCCCCC
24361	agcgcgcaag	tcccctttca	gaagacatgc	atgacccaga	tgcctgtgat	gagggtaaac
24421	cantontean	tratrarcar	chaacccgat	ggctgggcac	cgactctccc	caggatttag
24421	cageggeeag	cgacgagcag	-t	5900955000	agtococce	anatatatta
24481	aagagcgtcg	caagcttatg	atggccgtgg	tgetggttae	cgtagaacta	gagigicite
24541	gacatttett	taccgattca	gaaaccttgc	gcaaactcga	agagaatctg	cactacactt
24601	550500000	atttataaaa	carretre	aratatrtaa	cgtggaactc	accaacctoo
246UI	ttagacacgg	citiguada	caggcatgca	agatatttaa	cgtggaattt	accaaccigg
24661	tttcctacat	gggtattctg	catgagaatc	gcctaggaca	aagcgtgctg	cacagcaccc
24721	ttaaqqqqqa	agecegeget	gattacatcc	acaattatat	ttatctctac	ctataccaca
24721			540040404	2224444	20220333	ctassaga
24/8I	cgtggcaaac	eggeargggr	granggrage	aatytttaya	agaacagaac	Ctgaaagagc
24841	taaacaagct	cttacagaaa	tctcttaagg	ttctgtggac	agggttcgac	gagcgcaccg
2/9/1	teacttecas	cctaggagag	ctcatcttcc	cagagggtct	cagggttact	ttgcgaaacg
24701	cogocooga			*****	tasstattta	atactacasa
24961	gactgcctga	ctttatgage	cagageacge	LLaacaattt	tegetette	accetygaac
25021	gctccggtat	cctgcccgcc	acctgctgcg	cactgccctc	cgactttgtg	cctctcacct
25081	accordaato	cccccacca	ctatogagte	actoctacct	gttccgtctg	accaactacc
25001	4009094409	atarastata	2+002903+0	taaaaaaaa	caacttacta	gagtgtcact
25141	teteetaeea	cicggaigig	accyayyacy	LyayLyaya	cggcttgctg	gagegeedee
25201	gccgctgcaa	tctgtgcacg	ccccaccggt	ccctagcttg	caacccccag	ttgatgagcg
25261	aaacccagat	aataggcacc	tttgaattgc	aaggccccag	cagccaaggc	gatgggtctt
25201	abactergas	224442222	atasaaaaa	magtatagec	ctccgcctac	ttacacaaat
72271	Clectyggca	aaytttaaaa	crgacecegg	gactgtggac	tereservan	tegegeaage
25381	ttgccccgga	agattaccac	ccctatgaaa	tcaagttcta	tgaggaccaa	tcacagcctc
25441	cgaaagccga	actttcggcc	tocotcatca	cccagggggc	aattctggcc	caattgcaag
25501	costcosses	atecedecaa	gaatttctac	traasasarr	taagggggtc	taccttgacc
2550I	CCacccaaaa	accegecaa	gaaccccac	teataaaggg		
25561	cccagaccgg	cgaggaactc	aacacaaggt	tccctcagga	tgtcccaacg	acgagaaagc
25621	aagaagttga	aggtgcagcc	gccgccccca	gaagatatgg	aggaagattg	ggacagtcag
25691	ucauauuaau	cadeadaada	agacagteta	gaggagagte	tggaggaaga	cagtttggag
23001	gcagaggaag	cggaggagga	ggacagocog	949944494	eggaggaaga	attataataa
25/41	gaggaaaacg	annannnana	ggaggtggaa	gaagtaaccg	CCyacaaaca	
	3 3 3 3	aggaggeaga	00 00			accacceced.
7280T	gctgcggaga	caagcaacag	cgctaccatc	tecgetecga	gtcgaggaac	ccggcggcgt
25801	gctgcggaga	caagcaacag	cgctaccatc	tecgetecga	gtcgaggaac	ccggcggcgt
25861	gctgcggaga cccagcagta	caagcaacag gatgggacga	cgctaccatc gaccggacgc	tccgctccga ttcccgaacc	gtcgaggaac caaccagcgc	ccggcggcgt ttccaagacc
25861 25921	gctgcggaga cccagcagta ggtaagaagg	caagcaacag gatgggacga atcggcaggg	cgctaccatc gaccggacgc atacaagtcc	tccgctccga ttcccgaacc tggcgggggc	gtcgaggaac caaccagcgc ataagaatgc	ccggcggcgt ttccaagacc catcatctcc
25861 25921	gctgcggaga cccagcagta ggtaagaagg	caagcaacag gatgggacga atcggcaggg	cgctaccatc gaccggacgc atacaagtcc	tccgctccga ttcccgaacc tggcgggggc	gtcgaggaac caaccagcgc ataagaatgc	ccggcggcgt ttccaagacc catcatctcc
25861 25921 25981	gctgcggaga cccagcagta ggtaagaagg tgcttgcatg	caagcaacag gatgggacga atcggcaggg agtgcggggg	cgctaccatc gaccggacgc atacaagtcc caacatatcc	tccgctccga ttcccgaacc tggcgggggc ttcacgcggc	gtcgaggaac caaccagcgc ataagaatgc gctacttgct	ccggcggcgt ttccaagacc catcatctcc attccaccat
25861 25921 25981 26041	gctgcggaga cccagcagta ggtaagaagg tgcttgcatg ggggtgaact	caagcaacag gatgggacga atcggcaggg agtgcggggg ttccgcgcaa	cgctaccatc gaccggacgc atacaagtcc caacatatcc tgttttgcat	tecgetecga ttecegaace tggeggggge tteaegegge tactaeegte	gtcgaggaac caaccagcgc ataagaatgc gctacttgct acctccacag	ccggcggcgt ttccaagacc catcatctcc attccaccat cccctactat
25861 25921 25981 26041 26101	gctgcggaga cccagcagta ggtaagaagg tgcttgcatg ggggtgaact agccagcaaa	caagcaacag gatgggacga atcggcaggg agtgcggggg ttccgcgcaa tcccggcagt	cgctaccatc gaccggacgc atacaagtcc caacatatcc tgttttgcat ctcgacagat	teegeteega tteeegaace tggeggggge tteaegegge tactaeegte aaagacageg	gtcgaggaac caaccagcgc ataagaatgc gctacttgct acctccacag gcggcgacct	ccggcggcgt ttccaagacc catcatctcc attccaccat cccetactat ccaacagaaa
25861 25921 25981 26041 26101	gctgcggaga cccagcagta ggtaagaagg tgcttgcatg ggggtgaact agccagcaaa	caagcaacag gatgggacga atcggcaggg agtgcggggg ttccgcgcaa tcccggcagt	cgctaccatc gaccggacgc atacaagtcc caacatatcc tgttttgcat ctcgacagat	teegeteega tteeegaace tggeggggge tteaegegge tactaeegte aaagacageg	gtcgaggaac caaccagcgc ataagaatgc gctacttgct acctccacag gcggcgacct	ccggcggcgt ttccaagacc catcatctcc attccaccat cccetactat ccaacagaaa
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30721	cattcaacaa	catactttct	ccatacttta	aaggggatgt	caaattttag	ctcctctcct
30721 30781	cgttcagcgg gtacccacaa	catactttct	ccatacttta	gatgaccaag	agagtccggc	tcagtgactc
30721 30781 30841	cgttcagcgg gtacccacaa cttcaaccct	catactttct tcttcatgtc gtctaccct	ccatacttta tttcttccca atgaagatga	gatgaccaag aagcacctcc	agagtccggc caacacccct	tcagtgactc
30721 30781 30841 30901	cgttcagcgg gtacccacaa cttcaaccct agggtttatt	catactttct tcttcatgtc gtctacccct tccccaaatg	ccatacttta tttcttccca atgaagatga gcttcacaca	gatgaccaag aagcacctcc aagcccagac	agagtccggc caacacccct ggagttctta	tcagtgactc ttataaaccc ctttaaaatg
30721 30781 30841 30901 30961	cgttcagcgg gtacccacaa cttcaaccct agggtttatt tttaaccca	catactttct tcttcatgtc gtctacccct tccccaaatg ctaacaacca	ccatacttta tttcttccca atgaagatga gcttcacaca caggcggatc	gatgaccaag aagcacctcc aagcccagac tctacagcta	agagtccggc caacacccct ggagttctta aaagtgggag	tcagtgactc ttataaaccc ctttaaaatg ggggacttac
30721 30781 30841 30901 30961 31021	cgttcagcgg gtacccacaa cttcaaccct agggtttatt tttaacccca agtggatgac	catactttct tcttcatgtc gtctacccct tccccaaatg ctaacaacca actgatggta	ccatacttta tttcttccca atgaagatga gcttcacaca caggcggatc ccttacaaga	gatgaccaag aagcacctcc aagcccagac tctacagcta aaacatacgt	agagtccggc caacacccct ggagttctta aaagtgggag gctacagcac	tcagtgactc ttataaaccc ctttaaaatg ggggacttac ccattactaa
30721 30781 30841 30901 30961 31021 31081	cgttcagcgg gtacccacaa cttcaaccct agggtttatt tttaacccca agtggatgac aaataatcac	catactttct tcttcatgtc gtctacccct tccccaaatg ctaacaacca actgatggta tctgtagaac	ccatactta tttcttcca atgaagatga gcttcacaca caggcggatc ccttacaaga tatccattgg	gatgaccaag aagcacctcc aagcccagac tctacagcta aaacatacgt aaatggatta	agagteegge caacacecet ggagttetta aaagtgggag gctacagcae gaaactcaaa	tcagtgactc ttataaaccc ctttaaaatg ggggacttac ccattactaa acaataaact
30721 30781 30841 30901 30961 31021 31081	cgttcagcgg gtacccacaa cttcaaccct agggtttatt tttaaccca	catactttct tcttcatgtc gtctacccct tccccaaatg ctaacaacca actgatggta tctgtagaac	ccatactta tttcttcca atgaagatga gcttcacaca caggcggatc ccttacaaga tatccattgg	gatgaccaag aagcacctcc aagcccagac tctacagcta aaacatacgt aaatggatta	agagteegge caacacecet ggagttetta aaagtgggag gctacagcae gaaactcaaa	tcagtgactc ttataaaccc ctttaaaatg ggggacttac ccattactaa acaataaact

FIG. 28A-8

13201 tathacaca tatggacag gataaacc tcacctaac tytoaacty tygaaacca					taasaatssa	tatassita	togaaaacac
313121 chacqtqctc thagttggt tatcagacac tgtgaaccaa atgtcacac aaaagacaga 31381 aaacacaccaa thaagattat attttgactc thetggaaat cattactg atgaatcaga 31501 chacagcettt atgccaagaa chacagctta tecettcaac accactacta gggataatga 31561 aaactacatt catggaatat gttactacat gattattta tattagacta 31621 gaacatttca tatagcaaga cacagcettat gattettec aatgttgcct atgccagaag 31561 atttgaattg aatctaaatg cacagcagat tecagaaga aacatagcta ggtgacaca 31681 atttgaattg aatctaaatg cacagcagatt gattettec aatgttgcct atgccataca 31681 atttgaattg aatctaaatg cacagtgaat tecagaaga aacatagcta ggtgacaca 31801 thaaccaatc theteccacag cacagettta acatttgg taccattaga gatggacat 31801 thaaccaatc tectccacag cacagettta acatttgg taccattaga gatggacat 318181 tacaccaatc tectccacag cacagettta acatttgg taccattaga gatggacag 31981 aaaaatcat gegatagt tettaaagg etttacagag cattagattga gatggaa 32041 tecgggatet gggatcacgg tatetggaag cattacagag cattagattga gatggaga 32041 tecgggatet gggtcacag tettoggaag catgacttata tecgaaacg 32101 gtattggac attgtgtcc atcaggacac cacagcagc tgttetggc gggaccaga 32101 gtattggaca attgtgtca atcaatattgt traatacac ataatataga gtaggaaga 32101 gtattagaac atcagattat acaatattgt traatacac ataattaaa ggggtcaga 32201 taaatgacact tectgaag aggtcacag tgtcagag acagaatttc gatttcact aaattttg 3221 acacacatat tectgataa acacactac 32221 acacacactact tectgaaga aacacactac 32221 acacacactact tectgaagacac catgacacac ataacaagt traataaa 32221 tecggaacac acacgtcacc 32221 tecggaacac actgccaca acacacactac cacacactac gacacacactac gacacacactac gacacacactac gacacacactac gacacacacactac cacacactac cacacactac gacacacacactac acacactacac acacacactac cacacactac gacacacacactac gacacacacactac acacacactac acacacactac acacacac	31201	tattaacacc	ttatggactg	gaataaaccc	LLCaccidac	tyttaaatty	ttattaataa
313121 chacqtqctc thagttggt tatcagacac tgtgaaccaa atgtcacac aaaagacaga 31381 aaacacaccaa thaagattat attttgactc thetggaaat cattactg atgaatcaga 31501 chacagcettt atgccaagaa chacagctta tecettcaac accactacta gggataatga 31561 aaactacatt catggaatat gttactacat gattattta tattagacta 31621 gaacatttca tatagcaaga cacagcettat gattettec aatgttgcct atgccagaag 31561 atttgaattg aatctaaatg cacagcagat tecagaaga aacatagcta ggtgacaca 31681 atttgaattg aatctaaatg cacagcagatt gattettec aatgttgcct atgccataca 31681 atttgaattg aatctaaatg cacagtgaat tecagaaga aacatagcta ggtgacaca 31801 thaaccaatc theteccacag cacagettta acatttgg taccattaga gatggacat 31801 thaaccaatc tectccacag cacagettta acatttgg taccattaga gatggacat 318181 tacaccaatc tectccacag cacagettta acatttgg taccattaga gatggacag 31981 aaaaatcat gegatagt tettaaagg etttacagag cattagattga gatggaa 32041 tecgggatet gggatcacgg tatetggaag cattacagag cattagattga gatggaga 32041 tecgggatet gggtcacag tettoggaag catgacttata tecgaaacg 32101 gtattggac attgtgtcc atcaggacac cacagcagc tgttetggc gggaccaga 32101 gtattggaca attgtgtca atcaatattgt traatacac ataatataga gtaggaaga 32101 gtattagaac atcagattat acaatattgt traatacac ataattaaa ggggtcaga 32201 taaatgacact tectgaag aggtcacag tgtcagag acagaatttc gatttcact aaattttg 3221 acacacatat tectgataa acacactac 32221 acacacactact tectgaaga aacacactac 32221 acacacactact tectgaagacac catgacacac ataacaagt traataaa 32221 tecggaacac acacgtcacc 32221 tecggaacac actgccaca acacacactac cacacactac gacacacactac gacacacactac gacacacactac gacacacactac gacacacacactac cacacactac cacacactac gacacacacactac acacactacac acacacactac cacacactac gacacacacactac gacacacacactac acacacactac acacacactac acacacac	31261	taatacaaat	gatggcaaac	ttactttagt	actagtaaaa	aacggagggc	Ligitaatgg
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31561 agacattet cataggatat gitactacat gactagitat gatigaagic latticeal sile21 gacattette tataggetaa acagacgatat gatitetice aatgitigect algocataca 31681 attigaatig aatchaatig caagtgaate tectagaaage aacatageta cgctgaccaa 11741 atcoccotti tetetitette tacattacaa agacgacaac taaaataaag titaagtgit 31801 titaattaaa atcacaaaat tegagtagit attitigecte cacettecca tittaagtgit 31801 tetaaccaate tectoccace cacagtetta aacattigga acactataga agacgacaac taaataaag tittaagtgit 31921 gittiaagate cacaatacaa acacagittea aacattigga acacagitgi gagatacaga 11921 gittiaagate cacaatacaa acacagittea agacgageca atciggigga aggatagaa 21010 gatacggag attitateca acaggitea agacgacga catgattita atagcetta 319211 gatacggag attitaggite attiggaga aggatagga gigaacagag gillol gatacggag attitaggaga acacattata acaatatig tectagaa aggatagga catgattita atagcetta 319221 acatcaacti tetggigeg tectagaga acacattata acaatatig taataaaca acacattati acaatatig titaataaac atcigagacaa acacattati acaatatig titaataaca atcigagacaa acacatatat acaatatig titaataaaca atcigacaac accigicacaa accigicacaaaaa acciacaacaa giciii gagagagaacaa atagaagaac acaacaacaa gacacaacaa gacacaacaa accigicacaaaaa acciacaacaa gacacaacaagaacaacaacaaaaaaaaaa	31441	cttaaaaatt	CCacttaaaa	ataaatttt	tacagegace	agogaaaotg	aaastaataa
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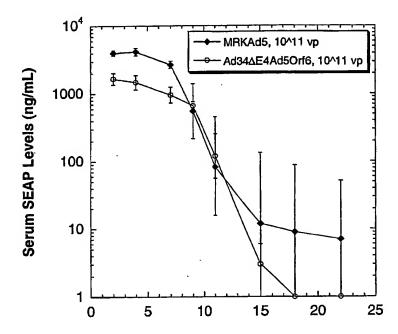


FIG. 29

Vaccine	Monkey	Pre		W	k 4	W	k 8	Wk	Wk 24		28	W	35
Wk 0, 4, 24	ID	Mock	Gag*	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
MRKAd5gag, 10 ⁴ 1 vp MRKAd5gag, 10 ⁴ 11 vp MRKAd5gag, 10 ⁴ 11 vp	00C018 00C034 00C058	1 0 4	5 4 4	13 5 3	1025 219 1086	0 5 0	824 404 440	8 3 4	756 445 1439	D 3 0	474 539 2338	0	383 216 940
Ad34AE1gagAE4Ad5Orf6, 10*11 vp Ad34AE1gagAE4Ad5Orf6, 10*11 vp Ad34AE1gagAE4Ad5Orf6, 10*11 vp	000038 000042 000066	6 6 3	8 30 18	5 4 1	111 89 118	1 4 1	301 264 816	0 1 0	224 73 429	1 0 0	535 181 439	0 0	233 69 273

Vaccine	Monk ID	•	D4 ⁺ CD3 ⁺ mphocytes	IFN-γ ⁺ CD8 ⁺ CD3 ⁺ per 10 ⁶ Lymphocytes		
		Mock	Gag ^a	Mock	Gag ^a	
Ad34ΔE1gagΔE4Ad5Orf6	00D038	22	154	130	450	
5 5	00D042	32	118	96	171	
•	00D066	12	238	150	442	

Vector	Vaccine	Monkey	P	10	T=4	wks	ts T=0 wks		Ts24 wks		T=26 wks		T=32 w/ca	
T=0, 4 wks	T=24 with	to	Mock	Cing*	Mock	Gag	Mock	dig	Mock	Cag	Mock	Gag	Mock	Cang
Ad34AE1gagAE4Ad5Od8, 10*11 vp	Ad35&E1gag&E4Ad5Orf8, 10^10 vp	000016	4	6	٦.	84	5	334	5	29	0	305	3	244
Ad34AE1gag&E4Ad3Oxf6, 10*11 vp	Ad35&E1gag&E4Ad5Orf8, 10^10 vp	000044	1	1	8	79	0	374	8	136	0	493	1	253
Ad346E1gag6E4Ad5Orf6, 10^11 vp	Ad35&E1gag&E4Ad5Ort6, 10*10 vp	00D064	1	8	1	125	8	655	6	145	٥	351	1	235
Natva	· · · · · · · · · · · · · · · · · · ·	00D087	1	1.	3	3	8	54	6	В	5	5	3	0

Vaccine (T=0, 4 Wks)	Vaccine (T=24 Wk)	Monkey		D4*CD3* mphocytes	IFN-γ*CD8*CD3* per 10 ⁸ Lymphocytes		
		ID	Mock	Gag	Mock	Gag	
Ad34AE1gagAE4Ad5Orl6, 10^11 vp	Ad35AE1gagAE4Ad5Orf6, 10^10 vp	00D016	62	433	176	1288	
Ad34∆E1gag∆E4Ad5Orf6, 10^11 vp	Ad35AE1gagAE4Ad5Orf6, 10^10 vp	00D044	136	593	323	1871	
Ad34AE1gagAE4Ad5Orf6, 10^11 vp	Ad35AE1gagAE4Ad5Orf6, 10^10 vp	00D064	188	785	292	892	

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